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BIOLOGICAL BULLETIN

SPERM-TRANSFER ORGANS IN CAMBAROIDES.

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Amongst the arthropods it is not uncommon for some of the limbs to be used for transferring the sperm from the male to the female. In some of these cases the path of the sperm from the male to the egg is very complex, and we may speak of "indirect sperm-transfer."

Thus in the common crayfishes of the United States, of the genus *Cambarus*, the first and the second limbs of the abdomen of the male are special organs that we will call the first and the second stylets, which conduct the sperm from the male openings upon the bases of the fifth pair of walking legs to the surface of the female. In the female the sperm is received into a pouch in the shell on the under side, between the fourth pair of walking legs. This sperm receptacle is hollowed out in the so-called annulus, or special sternal plate, and in this pouch the sperm remains till the eggs are laid. In the crayfish of Europe, however, the sperm conveyed by the stylets is deposited freely over the sternum, in secreted tubules, or spermatophores, and there is no sperm receptacle.

There are then two forms of indirect sperm transfer within this family, in the two genera *Cambarus* and *Astacus*.

The crayfish of Japan and the Amoor River region are so different from other species of *Astacus* that it is a question whether they do not form a distinct genus. They resemble *Cambarus* so much that Faxon called them *Cambaroides*. Nothing is known as to the method of sperm transfer in this *Cambaroides* subgroup of *Astacus*, but the following account of the anatomy of the organs concerned may aid in a tentative view as to what actual observation of the process may reveal.

The material used was kindly loaned by the National Museum,

and consisted of a very few specimens of *Astacus* (*Cambaroides*) *similis* from Corea and some ten specimens of *Astacus* (*Cambaroides*) *Japonicus* from Hakodate, Japan. These last were obtained by the "Albatross" from the market in July, 1906, and were remarkable in being all strung along upon bits of stick that had been thrust through several crayfish, one after the other, passing through the head-thorax and abdomen lengthwise. From the condition of internal anatomy the specimens would appear to have been dried before they were preserved by the naturalist.

Observations upon *Cambarus* have shown that when the stylets are being used to fill the sperm receptacle the male is firmly fastened to the female by two pairs of hooks, or spines, that stand out like spurs from the walking legs and are carefully fitted into the groove between the segments of the legs of the female. In fact, experiments show that without these spines the

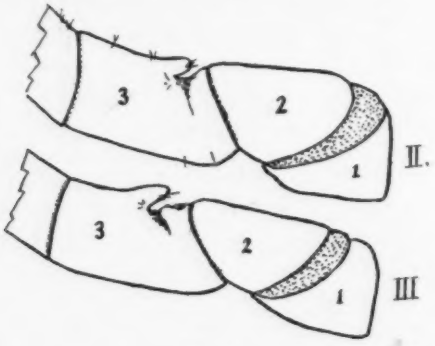


FIG. 1.

other organs are of no avail as the male is not able to transfer the sperm to the sperm pouch. In *Cambarus* there are then three sets of necessary external organs concerned in sperm transfer, the hooks and the stylets of the males and the sperm receptacles of the females.

In *Cambaroides* the hooks are present as well as the stylets but in the females there is no discovered receptacle though the annular plate is somewhat modified.

We will describe these three sets of organs, in the following order: hooks, stylets, annular plate of female.

In *Cambaroides similis*, a specimen 55 mm. long had the hooks developed as in Fig. 1, a blunt rounded spine upon the third segment of the second and the third walking legs. Each spine

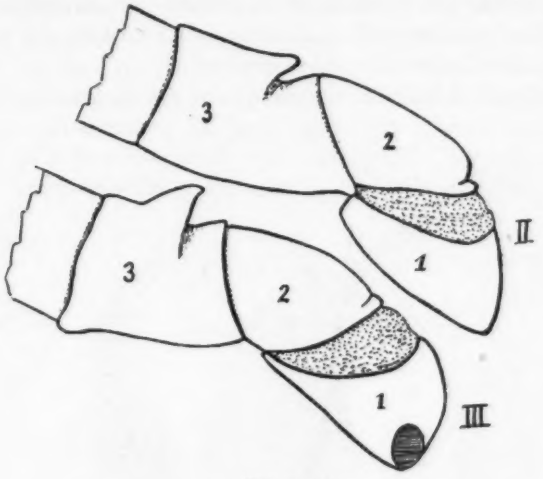


FIG. 2.

bears a few setæ. They are like the spines of *Cambarus Montezumæ* in being on the second and third legs, but are much shorter, more blunt and less effective as hooks. The spine of the third leg is the better developed.

In *Cambaroides Japonicus*, Fig. 2, the hooks are essentially the same, but more pointed. In both cases the resemblance of the hooks to those used by *Cambarus* is so strong that one would infer that they are probably functional in *Cambaroides*.

In comparing the stylets of *Cambaroides* with those of *Cambarus* we note that the first lacks the fine detail of apex commonly found in *Cambarus* and is a more stout and undifferentiated organ. In *Cambaroides similis*, Fig. 3, the first stylet is a clumsy cylinder having a movable joint between the long protopodite and the somewhat longer distal endopodite.

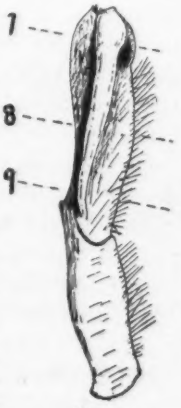


FIG. 3.

Both bear a fringe of setæ along the external edge. This long line of setæ seems to represent what is found upon the edges of the common pleopods and may be regarded as a mark of little specialization or of retention of primitive characters, since in *Cambarus* it is generally specialized as a local group of setæ, or at most in *C. Clarkii*, as a less simple line.

The main features of the endopodite are, however, a shallow groove and a very stout ridge along the posterior face, external to the groove. This ridge ends distally as a swelling that is part of the specialized tip of the organ. More enlarged, Fig. 4,

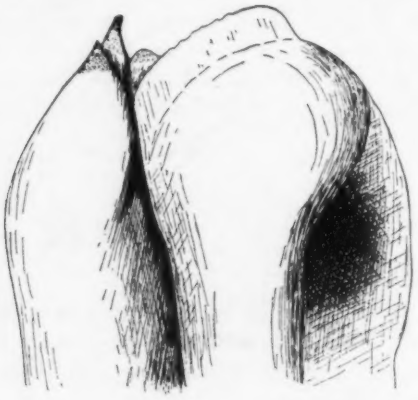


FIG. 4.

the tip shows the rounded head in which the ridge ends, the groove to the left of it and a depression to the right. The actual ending of the organ is a sort of complex edge, flattened from before back.

On the median side of the groove are two horny points, the shorter posterior, the longer anterior. The posterior is comparable to the large "spatula" and the anterior to the large "canula" of *Cambarus Montezumæ*. External to the groove is a short, blunt anterior point, comparable to the "ligula" of *C. Montezumæ* and a long thin knife edge that is a continuation of the rounded head of the ridge. The organ appears fitted to open a slit into which sperm might flow from the groove.

In *C. Japonicus* the two first stylets lie side by side with diver-

gent tips. In a male 45 mm. long, with stylets 8 mm. long, their tips were two millimeters apart, across the median line. Each stylet has the same structure as in *C. similis*, with only slight differences in proportion, but the groove opens more toward the median face and is not seen from behind, Fig. 5. While the tip Fig. 6 presents the same details as in *C. similis*, Fig. 4, the cutting edge is less sharply set off from the head of

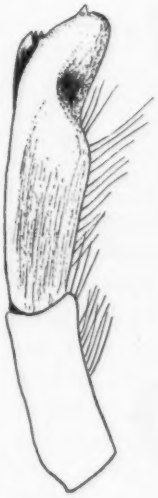


FIG. 5.

FIG. 6.

the ridge and runs out externally as a pronounced angle or spine, that is lacking in the former species.

From an end view the tip of the organ of *C. Japonicus* is complexly modelled and suggest not a cutting tool but a probe to be forcefully inserted against resistance.

Serial sections of the first stylets of both species show the same inside structure. A thick, firm shell covers the soft

areolar tissue and a delicate epidermis underlies the shell and is continuous with the areolar tissue. The internal anatomy and the external modelling is shown by the series of sections, Figs. 7, 8, 9, cut along the lines 7, 8, 9 of Fig. 3. The organ is essentially a thick, flat plate with a groove on its posterior face dividing it into a smaller median part that we will call the median mass and a larger external mass, *M.M.* and *Ex.m.* in Fig. 7.

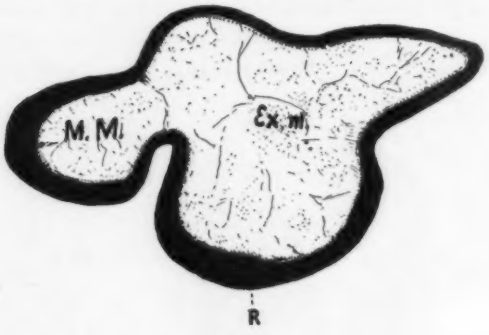


FIG. 7.

The groove is made much deeper by the fact that a great ridge, *R*, rises up from the external mass and forms the external boundary of the groove. In the middle of the course of the groove the ridge, Fig. 8, extends toward the middle line of the

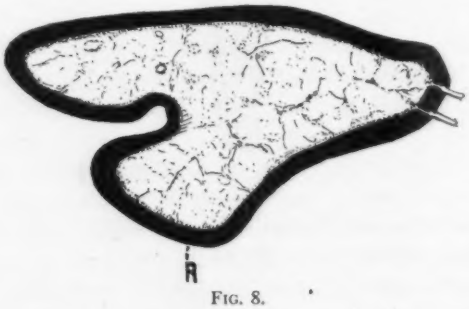


FIG. 8.

body parallel to the median mass so that the groove is here deep and narrow and opens out more to the median face of the organ. Toward the basal end, Fig. 9, the shallow groove is bounded by

the diminishing terminal part of the ridge. At this level also may be seen the muscle mass that extends into the endopodite and indicates that the joint between the endopodite and exopodite may actually be used and the position of the tip of the organ be

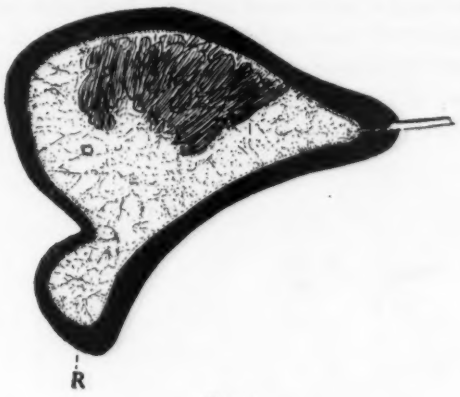


FIG. 9.

directed by this muscle. This muscle was also seen in cleared mounts in toto.

In the same manner in *C. Japonicus* sections show only a simple groove and large ridge, with the only difference that the groove faces more toward the median aspect of the organ, so that the above Figs. 7, 8 and 9 would well represent the condition in both species.

The second stylet preserves the usual pleopod form in that it is forked, or has both endopodite and exopodite. In *Astacus* (*Cambaroides*) *Japonicus*, Fig. 10, the exopodite is a slender obscurely segmented filament bearing few long setæ while the endopodite is the wide massive terminal part of the stylet. The tip of this endopodite, the flabellum, bears a long tuft of setæ and is evidently like the tip of the exopodite, but much enlarged. In the median edge of the endopodite there arises the extra element comparable to the "triangle" of *Cambarus*, that probably has some use in sperm transfer. This is a thick ridge that rises up as a free, thumb-like process directed diagonally across the endopodite. It has a marked angular elbow on the median side

and terminates in an oblique and somewhat hollowed face posterior to the flabellum. This very simple representative of the triangle of *Cambarus* and the scroll of the American and European *Astacus*, bears still a few setæ, several upon the median and two or three upon the external border. In this it is intermediate between the above two crayfish.

The entire appendage seems crude and clumsy, either primitive or reduced.

This appears again in the other species of *Cambaroides*, *C.*

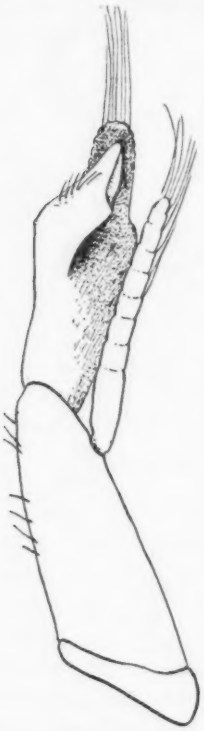


FIG. 10.

FIG. 11.

similis, Fig. 11, which is like *Japonicus* but the setæ are very short and the flabellum more reduced. The dotted area in Fig. 11 is membranous. The posterior face is turned so that the

triangle can fit into the groove in the first stylet. In *Cambarus* the projection of the second stylet fits accurately to the groove of the first stylet and insures sperm transfer and in *Cambaroides* we can see that the projection upon the second will run in the groove of the first but it does not seem nicely adjusted to it.

Another departure from the finer adjustments of *Cambarus* may be inferred from the simpler mode of ending of the defferent duct. While in *Cambarus* it ends in a soft papilla that is fitted

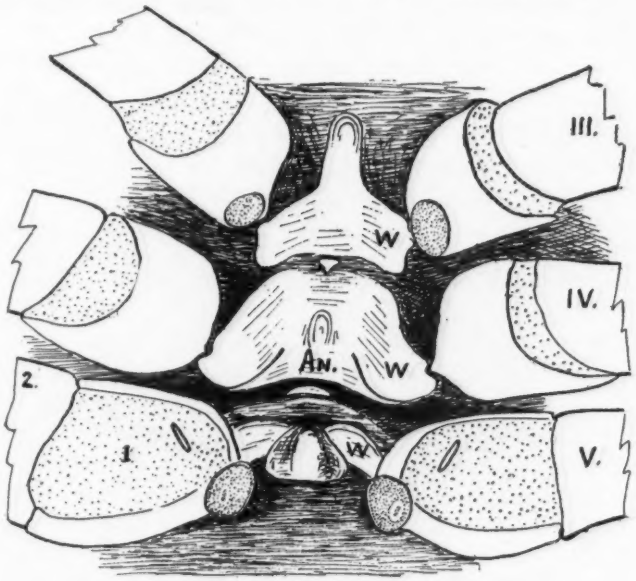


FIG. 12.

into the groove of the first stylet, in *Cambaroides* there is, at least in the preserved specimens, only a rounded, slightly raised area with a slit in it for the exit of the sperm.

The under side of the thorax of the male, Fig. 12, shows the ending of the defferent duct as a small opening in a rounded raised area on the base of the fifth leg, right and left. In *Cambaroides Japonicus*, Fig. 12, these rounded areas are soft and the entire adjacent surface of the base of the leg is also membranous, as indicated by the dotted region, except for the minute hard

oblique ridge isolated in the membranous area. In *Cambaroides similis*, the base of the fifth leg shows a large white area thought to be glandular, the area 1 in Fig. 13, and the ending of the defferent duct is in a solid projection with a slit-like orifice.

Whether in life a soft papilla can be projected from these

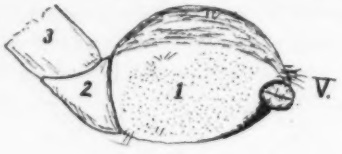


FIG. 13.

orifices is doubtful, but the hard and slightly projecting areas can be put against the groove in the first stylet in such a way that we infer the sperm may be poured out into the groove of the stylet, with perhaps some aid from the second stylet.

Turning now to the destination of the sperm transferred by the

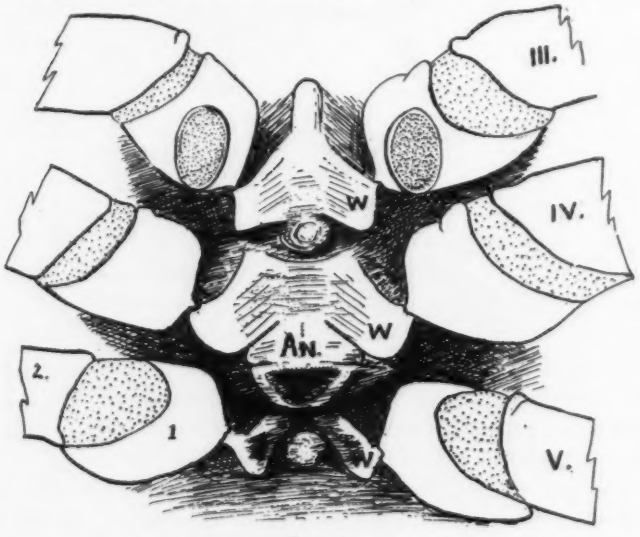


FIG. 14.

above male organs we fail to find upon the female any specialized receptacle. The under side of the thorax of the female *Cambaroides Japonicus*, Fig. 14, has a series of median plates between

the limbs *III*, *IV* and *V*. Each plate expands toward the limbs as a lateral wing, *W*, each plate is also modified in its central part. Between the third limbs the central part shows a rounded boss and an anterior prolongation; between the fifth limbs there is only a boss; between the fourth limbs the median part of the plate is prolonged backward not as a boss but as a large expanse, the annular plate, *An*. This annular plate is subdivided into an anterior part, *An*, somewhat convex from side to side and a posterior part which is hollowed out as is poorly shown in Fig. 14.

The hollowed posterior part of the annular plate rises up dorsally at an angle with the horizontal anterior part.

The median depression is quite shallow and neither in surface views nor in sections is there any slit or internal pocket such as is characteristic of the annular plate of the genus *Cambarus*. Thus while in both *Cambarus* and *Cambaroides* there is an annular plate only in *Cambarus* is it provided with an internal cavity. It is the internal cavity in the annular plate that is filled with sperm. In these specimens of *Cambaroides* no sperm pocket is found.

In the male *Cambaroides*, Fig. 12, there is also an annular plate between the fourth limbs but it lacks the hollowed posterior part that is found in the female.[1]

In a specimen of *Cambaroides similis* the posterior part of the annular plate is less sharply hollowed out than in *C. Japonicus*.

The use of these various male and female parts can, as yet, only be inferred from comparisons with the organs of *Astacus* and of *Cambarus* whose use has been observed. But in applying the male to the female we are led to imagine that the stylets may deposit spermatophores between the fourth and fifth limbs.

[1] In the individual male figured here there is an abnormal pair of structures that simulate the openings of oviducts. In the female the oviduct openings, Fig. 14, are large elliptical membranous areas upon the bases of the third limbs. In the male the openings of the defferent ducts are more elevated areas upon the bases of the fifth limbs, Fig. 13. There is thus both a difference in position and in character between the male and the female openings. The abnormal openings upon the third legs of the male figured in 12, are like female openings both in position and character though they are smaller than normal and the one upon the right of the animal i_s especially small (see also Fig. 2). This then seems to be a case of partial mixture of sex organs such as have been described in both *Astacus* and *Cambarus* (see *Am. Nat.*, 1909).

On the whole the sex organs of *Cambaroides* are more like those of *Cambarus* than like those of *Astacus*. That is, the hooks are present in *Cambarus* and *Cambaroides*, but not in *Astacus*. From their form we may infer that in *Cambaroides* they are used to hold the female just as they are used in *Cambarus*. The stylets in *Cambaroides* lack the flat scroll form of *Astacus* and are more like the stout complexly tipped organs of *Cambarus*, but they are much more simple. They doubtless serve to transfer the sperm as in both *Astacus* and *Cambarus*. In the female the annular plate of *Cambaroides* lacks the special sperm reservoir of *Cambarus* and is thus like *Astacus*, but it is more developed and somewhat hollowed out. In this respect it recalls the earliest phase of the ontogeny of the annulus of *Cambarus*.[1]

In brief the organs of *Cambaroides* are more simple than those of *Cambarus* but fashioned somewhat like them, suggesting some connection closer with *Cambarus* than with *Astacus*.

How are these facts to be interpreted? The general anatomy shows that *Cambarus* is the recent and *Astacus* the more unspecialized genus. Is *Cambaroides* a step from *Astacus* toward *Cambarus* or is it a step backward from *Cambarus*?

Since *Cambaroides* has the same gill formula as *Astacus* and is like *Astacus* in having no sperm receptacle (as far as known), while on the other hand it has hooks like *Cambarus* and stylets similar to those of *Cambarus*, we may regard it as a genus separate from both *Astacus* and *Cambarus*.

It then becomes a question of the relative positions of these three genera. Granting that the larger number of gills is primitive and the small number derived we must assume either that the presence of hooks in *Cambarus* with few gills and in *Cambaroides* with more gills is a case of secondary convergence from parallel variation or else that it is a common inheritance. Ortmann has assumed the resemblances of *Cambarus* and *Cambaroides* due to convergence, but Faxon regarded them of more significance. The new facts as to the annular plate and the structure of the stylets will aid in the solution of this question; with emphasis upon the sex organs as criteria of relationship, which has been the tendency of all recent work upon this group.

[1] Biol. Bull., X., Figs. 6, 7.

As elsewhere shown[1] some of the Penæidæ as well as the lobsters have sperm receptacles whence we may infer that a sperm receptacle was common to the ancestors of the crayfish. In such case the absence of sperm receptacle in *Astacus* would be due to loss, and the presence in *Cambarus* to retention of the ancestral mode of sperm transfer. If we suppose that the resemblances and differences of organisms are connected with chance variations it seems more likely that organs may have been independently lost, in separate animals, than that the same organ should have been independently acquired in separate organisms. We would then say that *Cambaroides* and *Cambarus* are closely related as both have retained the hooks and the general form of stylets of some ancestor but that *Astacus* and *Cambaroides* are not so closely related though both have independently lost the sperm receptacle while changing the form of stylets.

Adopting Ortmann's views as to the origin of the present distribution of crayfish we would modify it chiefly by the assumption of two migrations from Asia into America. We would think of ancestral Asiatic crayfish with many gills and a sperm receptacle of some sort filled by some use of the abdominal limbs. One set of descendants retaining more gills but losing the sperm receptacle became the *Astacus* of America and Europe as well as the crayfish of the southern hemisphere. While another set of descendants became *Cambarus* and *Cambaroides*. Part of this branch migrated into America and ultimately, in Mexico according to Ortmann's evidence, became the present *Cambarus* with reduced gills and highly specialized receptacle and stylets. The other part remaining in Asia independently lost the receptacle but retained the larger number of gills as well as the hooks.

We might then find in *Cambaroides* indications of the former presence of a sperm receptacle. As such we regard the hooks that have no known use except as aids for the filling of a receptacle. As such we regard the presence of ligula, spatula and canula at the tip of the stylet.

The absence of a tubule in the stylet, its clumsy form and the reduced prominence of its tip as well as the simplicity of the triangle of the second stylet might also be regarded as signs of degeneration.

But speculation is here very insecure and if use inheritance or some law of perfection were known it would be easy to argue that *Cambaroides* was an incipient *Cambarus* evolving from *Astacus*. In any case the common ancestor of *Cambarus* and *Cambaroides* must have been far back as it had the larger number of gills and as *Cambaroides* has the primitive characters of a well-developed flagellum on the first stylet and a muscle at the movable joint between protopodite and endopodite.

A diagram of the three genera would place *Astacus* and *Cambaroides* near together as having the same gill formula and as lacking a sperm receptacle, while *Cambarus* should stand apart as having a simplified gill formula and also very highly developed sperm-transfer organs, including a sperm receptacle. At the same time the diagram should indicate that *Cambarus* and *Cambaroides* kept together after departing from *Astacus*, and that later *Cambaroides* went off in the direction of *Astacus*, leaving *Cambarus* as at once the most specialized in its gills and the most conservative in its retention of the very ancient crustacean mode of sperm transfer by the employment of a sperm receptacle.

BALTIMORE, June 10, 1909.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL.

III. A Case of Incomplete Hermaphroditism.[1]

RAYMOND PEARL AND MAYNIE R. CURTIS

Origin and General Character of Specimen.

From a chick hatched in the spring of 1907, at the Maine Agricultural Experiment Station, there developed the bird which forms the subject of this paper. This bird was a Barred Plymouth Rock and when adult presented externally the general appearance of a normal hen of this variety, so far as the characters body form and plumage color were concerned (cf. Plate I.). As the photograph in Plate I. shows, however, the head and neck resembled these parts in a cockerel. This resemblance was especially remarkable in respect to the size and shape of the comb and wattles. The comb was obviously much larger than the comb of a normal Barred Plymouth Rock hen and looked exactly like the comb of a male bird. This was also true of the wattles.

The dimensions[2] of the comb of this bird were as follows :

Length	88.4 mm.
Calculated height	25.1 mm.
Area	22.2 cm.^{2}

For normal adult Barred Plymouth Rock females the following average values for comb size have been found :[3]

Mean length	50.80 $\pm$ .56 mm.
" calculated height	10.57 $\pm$ .23 "
" area	5.59 $\pm$ .17 cm.^{2}

It is evident from these figures that the comb in this specimen greatly exceeds in size the average for females of the variety.

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 13.

[2] Made in accordance with the methods described by R. and M. D. Pearl in a paper "Data on Variation in the Comb of the Domestic Fowl," *Biometrika*, Vol. VI., pp. 421–423.

[3] Pearl, R. and M. D., *loc. cit.*, p. 427.

In regard to behavior this bird resembled a normal hen rather more than a cock. She was never heard to cluck, however, or to make any of the sounds which normal active hens make in the course of the day's work. This bird probably never laid an egg, though we are unfortunately not able to make an absolute statement on this point. The egg records of the station show an egg to the credit of this bird on November 7, 1907. This was the only egg ever recorded for this bird, and it is undoubtedly an erroneous record. As will presently appear, the condition of the sexual organs was not such as to indicate that they had ever been functional.

Cockerels placed in the pen with this bird would try to fight with her as if she were a cockerel; but she would not fight.

FIG. 1. Outline of the lateral aspect of the comb of the Barred Plymouth Rock hen described in this paper. This outline is actual size.

We have no evidence that a cockerel ever attempted copulation with this bird. These facts are of interest in relation to the question of the basis of sex-recognition and the assortative mating known to occur among fowls. Is a normal pullet with an unusually large comb less likely to have her eggs fertilized than a bird with a smaller comb?

This bird was observed occasionally to take the position of a cockerel about to crow and attempted to crow but never succeeded in very closely approximating the sound of a normal cock bird. The bird was never seen to attempt to tread a hen.

Autopsy.

The appearance and behavior of this bird led to the suspicion that it represented a case of true hermaphroditism. On August 24, 1908, the bird was killed and a post mortem examination made. The weight of the body after bleeding was 2,725 grams. The body cavity contained much fat. The alimentary tract and attached viscera were entirely normal. The following measurements were made:

From gizzard to origin of cœca	167 cm.
Longest cœcum	22 "
From origin of cœca to cloaca	13 "

The following weights were taken:

Gizzard	125 gms.
Liver	44 "
Heart	9.5 "
Spleen	4.5 "

On the left side of the body was a normal oviduct. There was an ovary in the usual position. It was of about the size of the ovary of a laying hen after the removal of large yolks. It had a coarsely granular appearance and showed many folds. There were no eggs visible and its surface did not have the ragged appearance, due to ruptured follicles, which is characteristic of the ovaries of laying hens. The rest of the urinogenital system was completely covered by fat. The part of the body containing this organ system was hardened in formalin for further dissection.

Gross Anatomy of Reproductive Organs.

Dissection confirmed the suspicion of hermaphroditism. On the left side were the female-like reproductive organs described above while on the right side there was a set of organs similar to those of the normal male. The gross anatomy of the reproductive system in this bird is shown in Plate II., Fig. 1.

The female organs were more nearly normal than the male. The ovary, like a normal ovary, was ventral to the craniad lobe of the left kidney, covering, when viewed from the ventral side, all but the caudo-lateral angle of this lobe. It extended past the cranial margin of the kidney in the hermaphrodite nearly to, and in the normal hen with which comparisons were made, slightly beyond the fourth rib. In the hermaphrodite the ovary

was developed less on the medial side than in the normal hen. The measurements of greatest length and breadth of ovary in the hermaphrodite were 29 mm. by 16 mm. while in the normal hen they were, excluding the projecting yolks, 34 mm. by 20 mm. The ovary was attached to the body wall near the middle line by a thick stalk-like portion. This appeared perfectly normal. Its longest dimension (cranio-caudal) was 15 mm. compared to 18 mm. in the normal bird. The external appearance of the ovary was quite different from that of a normal ovary. It seemed to be a coarsely granular but otherwise homogeneous mass covered by peritoneum and minutely and very irregularly folded. It did not have the ragged appearance of the normal ovary and the minute folded masses did not look like the yolks of similar size in the normal ovary. They seemed to be folds on the surface of a homogeneous mass rather than small spheres of yolk enclosed in follicles.

The oviduct was normal in appearance and in position. The mouth of the funnel faced the ovary while the cranial ends of the lips were fused and extended across the left kidney to the fourth thoracic rib some distance laterad of the cranial end of the ovary. The caudal ends of the lips also fused and were attached to the ventio-median margin of the ligament which holds the convolutions of the oviduct in place. The oviduct presented the same principal convolutions as a normal active oviduct. It was larger than the oviduct of the adult hens we have examined which had never laid and the ovaries of which did not show a number of yolks. Table I. gives the lengths of the oviducts we have been able to examine in this condition.

TABLE I.

Dimensions of Oviducts of Pullets which have Never Laid and which have no Growing Yolks on Ovary. Hermaphrodite Included for Comparison.

Band Number of Bird.	Length of Oviduct in cm.	No. of Yolks Above 1 cm. on Ovary.	Total Number of Eggs Laid.
Hermaphrodite			
16 D	47.0	0	0
129 E	13.0	0	0
297 E	20.0	0	0
222 E	23.5	0	0

The length of the oviduct of the hermaphrodite hen 16 as

given in the first line of this table is 47.0 cm. This is just twice the length of the next shorter oviduct, that of hen 222 E. The oviduct is however smaller than in hens actively engaged in egg production in the middle of a laying period. It compares most nearly in size with the oviduct in hens which have ceased laying from 5 to 16 days before examination, or to those with 4 to 6 large yolks on the ovary. In order that this comparison may be readily made Table II. is introduced. This table is compiled from records in the archives of the laboratory and gives certain data in regard to normal hens having oviducts between 44.5 cm. and 50.5 cm. long, *i. e.*, of approximately the same length as that of the hermaphrodite hen No. 16. The table includes the following data: (*a*) length of oviduct in cm.; (*b*) the number of yolks 1 cm. or more in diameter on the ovary at the time of autopsy; (*c*) the number of days elapsed since the last egg was laid. In case the bird never laid the sign ∞ is used to denote this fact.

TABLE II.

Data from Normal Hens on Oviducts of Same Size as that of Hermaphrodite Bird.

Band Number of Bird.	Laboratory Autopsy Number.	Length of Oviduct in cm.	Number of Eggs 1 cm. or More in Diameter on Ovary.	Number of Days Since Last Egg Was Laid.
Hermaphrodite				
16	175	47.0	0	∞ (?)
260 D	117	47.0	0	8
15 D	124	50.0	0	5
213 D	133	46.0	0	7
788 D	137	45.0	0	9
38 D	151	46.0	0	8
405 D	171	50.0	4	9
317 E	179	50.0	4	∞
480 E	202	49.0	2 absorbing	16
314 E	236	48.5	2 "	10
96 E	237	49.0	6 "	∞
429 E	248	48.5	14 (hard, absorbing)	9

From the table it is apparent that the oviduct in the hermaphrodite hen was in essentially the same condition as that of a normal hen which has recently completed an egg-laying period (clutch).

Internally the oviduct of the hermaphrodite was differentiated into the regions characteristic of the normal oviduct. The funnel walls were thin and transparent. Grandular ridges appeared

at the end of the funnel and became gradually heavier and higher. In the albumen-secreting portion of the tube they were heavy, high and irregularly lobed. The ridges at the cranial end of the isthmus were thin and straight but did not preserve that character so strictly as in a normal oviduct, so that near the shell gland they resembled the ridges in the albumen-secreting portion. The shell gland ridges were high, very irregular and much lobed. These turned dark in the preserving fluid as we have often noticed to be the case with normal oviducts. The vagina had the characteristic low straight ridges. The dimensions of the various parts of the oviduct were as follows:

Length of funnel neck	2.5 cm.
Width of flattened tube at point where funnel passes into albumen portion	0.6 "
Length of albumen portion	18.5 "
Width of flattened tube at widest part of albumen portion	0.9 "
Length of isthmus	7.0 "
Length of shell gland	7.5 "
Width of widest part of shell gland	2.0 "
Length of vagina	11.5 "

The opening of the oviduct was in the normal position, slightly to the left of the midventral line. The margin of the opening was folded, but was inconspicuous while in a laying hen it protrudes a little into the cloaca. A large probe was passed from the vagina into the cloaca demonstrating a natural opening between these organs.

The left suprarenal body was covered by the cranial end of the ovary as in normal cases.

Directly opposite the middle of the ovary on the left side of the body was a small irregular, though generally ovoid organ, the testis (Plate II., *T*). This organ was 9 mm. in length by 6 mm. in greatest breadth. It was attached to the body wall by its broad side with the more convex side median and the nearly straight side lateral. This organ did not appear macroscopically like a testis but looked to the naked eye or through the hand lens, like a small mass of the same sort of tissue as the ovary already described, but covered with an additional layer of connective tissue which obscured the minute foldings.

From the lateral side of the testis a duct passed to the cloaca

running parallel to the median line and ventral and lateral to the ureter. This tube was nearly straight throughout its course, but had a few convolutions near the cloacal end. It had the position and appearance of a normal vas deferens in a young cockerel. The tube was heavy walled and gradually increased in diameter caudad. Sections showed that this duct had a definite lumen. There was no enlargement comparable to a seminal vesicle. It was not possible to demonstrate an opening into the cloaca.

Histology of the Left Genital Gland (Ovary).

The left genital gland was much less finely lobulated than a normal ovary. The large lobules had smooth contours. The organ was covered with a layer of peritoneum. Over most of the surface the cells of this layer were nearly cubical but in some portions they were shorter than broad while in other regions they were nearly twice as tall as broad. Over a few small areas there was an outward proliferation of this epithelium so that evaginated folds of the epithelium four to six cells deep projected from the surface. In a few cases these evaginated ridges were still further folded along their lateral margins.

Beneath the peritoneal layer was the tissue which formed the bulk of the organ. This was a highly cellular but much vacuolated tissue, the cells of which were not unlike the cells of the stroma of a young ovary. This tissue was nearly uniform throughout the organ. In the vacuoles of this tissue were found, in many portions of the organ, irregular non-cellular masses which stained deeply with acid stains, especially eosin. Some of these masses were surrounded by a single layer of very much flattened cells. They did not appear like ova nor did the surrounding cells resemble normal follicle cells. In the part of the organ ventral to the suprarenal body were a few spherical portions of the stroma-like tissue which were more dense and took a deeper stain with hæmatoxylin. These portions did not differ in other particulars from the surrounding tissue.

The stroma-like tissue contained few blood vessels but a highly vascular connective tissue penetrated the organ from the stalk. This tissue appeared like a core to the organ projecting into the larger lobules in tongue-shaped masses.

No Graaffian follicles or Pflüger's tubes were found, though series of sections from all parts of the gonad were examined. The general histological structure of the organ was such as to indicate that it was in a degenerating condition at the time the bird was killed. This process of degeneration had gone so far that nothing like normal ovarian tissue was to be found. Whether at any time in the life of the bird any part or all of the ovary had been normal in structure, it is impossible now to say. The condition of the gland at death afforded no certain evidence either for or against this view. That oögenesis, however, could not have gone beyond early stages during the later life of the bird is made probable by the fact that it did not lay (except for the single doubtful egg noted on p. 272), although possessed of a normal oviduct.

The net result of the microscopical examination of the left genital gland, which had the normal anatomical relations of an ovary, is negative.

Histology of the Right Genital Gland (Testis).

The limiting membrane of the right genital gland was not very thick and was poorly preserved in our sections. Such parts of it as were intact seemed to have a cellular outer layer with a fibrous inner layer. We could not be sure of futher histological details nor could we determine the extent of this tunic.

The gland contained no normal seminiferous tubules but showed evidence of tubular origin. The central portion was more dense than the periphery and in this more dense portion a few places showed the cells arranged as if small cellular rods had been cut in various planes. These rods might be considered tubes without lumen. They were formed by a single layer of nearly cubical cells, about the size and form of the epithelial cells of the seminiferous tubules at the age when these form a single layer nearly filling the lumen. Around these rods was a thin layer of fibrous tissue. Between the dense central portion and the periphery the epithelial cells gradually disappeared so that the greater portion of the gland appeared to be a connective tissue skeleton representing the basal membranes of the tubes, and the intertubular stroma of the young gland. Most of the tubes formed by the remaining basal membranes contained a few

cells but these were irregular and had lost their epithelial character. In some of the tubes were eosin-staining non-cellular masses like those found in the left genital gland. In the right gland we did not find cells surrounding these masses.

On the dorsal side of the organ was a mass of tubules. Those seen in each section varied considerably in diameter but they were much smaller toward the cranial end of the organ. This mass of tubules extended the full length of the testis. The tubes were lined with simple, heavily ciliated, columnar epithelium. Outside this, especially in the larger tubules, could be distinguished one or more layers of smooth circular muscle cells. The tubes were imbedded in connective tissue. This tubular structure was in all essential particulars precisely like a normal epididymis and without any question represents that organ. A photograph of a section through the epididymis is shown in Plate II., Fig. 2. The magnification used in this figure is low, but on the original negative the cilia on the cells lining the tubes can be plainly seen.

On the median side of the testis and lying for the most part at the side of the epididymis, though in some portions extending between the gland and the epididymis, was a mass of very vascular connective tissue.

Sections of the vas deferens at about the middle of its length showed it to be a tube considerably larger in diameter than the largest part of the epididymis. It was lined with columnar epithelium showing, in some sections, two rows of nuclei close together. In some sections cilia could be distinguished, but they were not so easily demonstrated as in the epididymis. There was a subepithelial layer of non-muscular tissue, probably the mucosa, and outside this a thick layer containing smooth muscle fibers. We were unable to distinguish different muscular layers in our sections.

The lumen of the vas deferens and also parts of the epididymis contained masses which stained strongly with eosin. These masses included irregular fragments that took the chromatin stain.

In general, the histological study of the right genital gland led to the same conclusion as did that of the left, namely, that we were dealing with a degenerating structure. As is indicated in the

foregoing description, however, the right gland approached somewhat more nearly to the normal than did the left. Whether this organ was even functional, however, in the sense of containing actively dividing spermatogonial cells, cannot be determined from the evidence afforded by the histology of the gland at death. It may or may not have been. One cannot tell. So then again our results on the question as to whether actual spermatogenesis occurred in this hermaphrodite fowl are negative. All that can be positively affirmed is that at the time when the bird was killed, both sexual glands were in an inactive and degenerating condition.

Discussion of Results.

The case above described presents a number of points of considerable theoretical interest which we may now, with the facts in hand, proceed briefly to discuss.

The first point to which we would direct attention is the peculiar combination or correlation of sexual characteristics (primary and secondary) which existed in this bird. Externally it presented a condition essentially similar to the rarely observed antero-posterior gynandromorphism of insects. Anteriorly the bird was male in its external somatic characters; posteriorly it was female. The truth of this statement may be demonstrated in a striking manner by placing the edge of an opaque card along a line connecting the letters *a* and *b* in Fig. 1 of Plate I. and turning the card about this edge as an axis so as to expose alternately the anterior and posterior parts of the bird. When the card covers the posterior part of the bird what one can see (*i. e.*, the anterior part) is unmistakably and indubitably male. On the contrary, when the anterior part is covered by the card, what of the bird is then to be seen is equally unmistakably female. The "maleness" and "femaleness" of these two portions of the body extend to the most minute details of structure, perhaps not apparent to anyone not perfectly familiar through first-hand practical experience with poultry and particularly Barred Plymouth Rocks. Thus the beak — which is not ordinarily reckoned as a secondary sexual character — in this bird is to the fancier unmistakably that of a male.

It is certainly a remarkable fact that with this perfectly clear

and definite somatic gynandromorphism there is associated an absolutely inactive condition of the primary sexual organs, so far as the functions of spermatogenesis and oögenesis are concerned. The case shows clearly enough that the secondary sexual characters of both sexes *may* exist without the accompaniment of functionating germinal epithelium in the same individual. It does *not* prove that the secondary characters may originally *develop* in the absence of the functioning of the primary glands, because of the uncertainty as to whether either of the glands was ever functional in this specimen.

There has accumulated in recent years a considerable mass of evidence,[1] particularly from medical, surgical and gynecological workers, tending to show that the development of secondary sexual characters is in some way controlled through internal secretions (containing hormones) produced in some part or parts of the primary sexual apparatus. While the general fact of such a relationship is now to be regarded as quite definitely established, the details of the process are as yet by no means worked out. Whether these secretions are elaborated in cells of the germinal epithelium proper, from interstitial or stromal cells, or from the accessory parts of the reproductive apparatus (*e. g.*, epididymis, oviduct, etc.) is, in general, still unknown. It might at first thought be supposed that the present case, inasmuch as the glands are degenerate and non-functional whereas the accessory male and female organs (epididymis, vas and oviduct) are complete and normal, afforded evidence in favor of the view that these latter organs are sources of internal secretions influencing secondary sexual characters. Any presumptive warrant for such an inference, however, is largely if not entirely taken away by evidence of another kind. We have conclusively shown, for example, in unpublished experimental work that complete or partial removal or ligation or section of the oviduct in the domestic fowl, undertaken before or after the oviduct has become functional, is without any effect whatever on the development or

[1] It seems unnecessary to print *in extenso* here the long list of literature which exists on this subject. An introduction to this literature will be found in Morgan's "Experimental Zoölogy," Chapters 28 and 29, and in Bayliss, W. M., and Starling, E. H., "Die chemische Koordination der Funktionen des Körpers," *Ergeb. der Physiol.*, Jahrg. V., pp. 664–697, 1906.

persistence of the female secondary sexual characters. The fact that in man vasectomy (practised, for example, in Indiana for the sterilization of criminals and certain other undesirable citizens) produces no effect whatever on secondary sexual characters or the sexual appetite is again evidence in the same direction.

The present case, of course, affords no direct evidence as to whether a secretion influencing secondary sexual characters may not be produced by the interstitial or stromal cells.

A further point of considerable interest lies in the fact that in this bird we have a fully developed, normal, and so far as can be told, entirely functional oviduct in the absence of a functional ovary. Normally in the hen the oviduct is in an atrophied, non-functional condition at times when laying is not going on, *i. e.*, when the ovary is not functioning. In the young pullet the oviduct stays in an infantile condition until the oöcytes begin to enlarge by the deposition of yolk just before laying begins. As the yolks approach the size at which they are separated from the ovary the albumen-secreting and other glands of the oviduct become enormously enlarged and the whole organ passes into the "laying condition." After laying stops the glands quickly atrophy and the whole organ goes back to the adolescent condition. In other words, there is in the normal bird a close correlation between the functioning of ovary and oviduct. There is, of course, a similar apparent correlation between ovary and uterus in mammals.[1] Now in this hermaphrodite specimen the correlation is apparently upset. We have the oviduct in "laying condition" in a bird in which the ovary is absolutely non-functional so far as ovulation is concerned. The two cases of hermaphroditism in the domestic fowl described by Shattock and Seligmann[2] essentially parallel ours in this regard. In both cases they found a well-developed

[1] Here the brilliant work of Dr. Leo Loeb is establishing, by means of analytical experimentation, the causal factors in the physiology of the uterus. Cf. Loeb, L., "The Production of Deciduomata and the Relation between the Ovaries and the Formation of the Decidua," *Jour. Amer. Med. Assoc.*, Vol. L., pp. 1897–1901. June 6, 1908.

[2] Shattock, S. G., and Seligmann, C. G., "An Example of True Hermaphroditism in the Domestic Fowl, with Remarks on the Phenomenon of Allopteratism," *Trans. Pathol. Soc. London*, Vol. 57, pp. 69–109, Plate I., 1906. "An Example of Incomplete Glandular Hermaphroditism in the Domestic Fowl," *Proc. Roy. Soc. Medicine*, Vol. I., pp. 3–7, 1907.

oviduct, though the ovary was distinctly not in *ovulating* condition.[1] These cases point strongly to the idea that the mutual interrelationship between ovary and oviduct in birds is very far from being of such a simple character as one would be led to infer from observation of normal specimens. Here, as in other instances, teratology may furnish the clue for the elucidation of a normal physiological process.

Summary.

The purpose of this paper is to describe in detail a case of incomplete hermaphroditism in the domestic fowl. It is shown that:

1. In its external somatic characters the specimen was an antero-posterior gynandromorph.

2. Internally the bird possessed on the left side a large, lobulated gland in the position and anatomical relations normal to the ovary. There was also a fully developed, normal oviduct, in functional condition on the left side of the body.

3. On the right side of the body was a small organ in the position and anatomical relations normal to the right testis. Attached to this organ was a normal epididymis and vas deferens leading to the cloaca.

4. Microscopical examination showed that both sex glands were in a condition of extreme degeneration. Neither spermatogenesis or oögenesis could be found in any part of either gland.

5. Certain theoretical aspects of the case are discussed.

[1] These authors did, in these cases, succeed in finding some evidence of actual oögenesis, but not of ovulation, either past or prospective.

Explanation of Plate I.

Showing the hermaphrodite specimen described in the text. A normal male and female of the Barred Plymouth Rock breed are shown for comparison.

Fig. 1. Hermaphrodite specimen. A line connecting the letters *a* and *b* marks the division region between the male and female portions of the gynandromorphic condition. Cf. text, p. 280.

Fig. 2. Normal Barred Plymouth Rock cockerel.

Fig. 3. Normal Barred Plymouth Rock pullet.

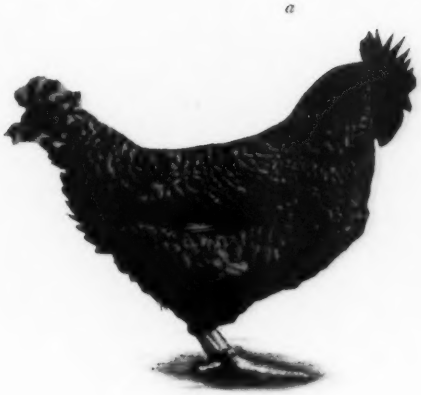


FIG 1

FIG. 2.

FIG. 3.

Explanation of Plate II.

Fig. 1. Photograph showing the gross anatomy of the genital organ of the hermaphrodite specimen. *O*, ovary. *F*, funnel mouth of oviduct (ostium tubæ abdominale). *S*, region of shell gland of oviduct. *T*, testis. *V.D.*, vas deferens. *U*, right ureter. A black card is placed behind the vas deferens and ureter in the lower portion.

Fig. 2. Microphotograph of section through epididymis. Obj.: Spencer 32 mm.; 6× compensating ocular.

FIG. 1.

FIG. 2.

FURTHER STUDIES ON THE LIFE CYCLE OF PARAMECIUM.

LORANDE LOSS WOODRUFF.

I. Introduction 287
II. Methods 288
III. Description of Cultures 292
IV. Discussion 295
V. Conclusions 306
VI. Literature 307

I. Introduction.

The life cycle of infusoria has been the subject of numerous investigations since Ehrenberg suggested on *a priori* grounds that the protozoa are so simply organized that they are not subject to natural death, and Dujardin opposed the view and maintained that the life history of infusoria comprises a cyclical change in vitality which terminates in death.

Bütschli ('76), Engelmann ('76), Maupas ('88; '89), Joukowsky ('98), Simpson ('01), Calkins ('02; '04), Woodruff ('05), Popoff ('07) and Gregory ('09) have all advanced evidence tending to show that infusoria when bred under somewhat constant culture conditions pass through a more or less definite physiological cycle. This cycle is characterized by an initial high potential of division which gradually is expended until reproduction finally ceases, and death puts an end to the cycle unless conjugation is permitted or artificial stimuli are employed. Characteristic morphological changes, both cytoplasmic and nuclear, appear in many cases as "senile degeneration" increases.

Enriques ('08) in a recent paper has again opposed the idea of old age and physiological death in protozoa and has contended that the results which support the cyclical character of the infusorian life history have been obtained by faulty culture methods. The conclusion of Enriques is, I believe, somewhat too sweeping, and is based in part on a misunderstanding of the methods by which the most extensive cultures have been con-

ducted. The work on the infusorian life history has clearly shown that many species of infusoria, when bred on a more or less constant culture medium, pass through quite definite cycles. Calkins, Woodruff, and Gregory have shown also that specific changes in the environment at critical times may "rejuvenate" a culture and lengthen its life for long periods. It is demonstrated, I believe, that the life history of the infusorian is cyclical when subjected to a constant environment, and it is also demonstrated that the life history may be lengthened by the timely use of various stimuli.

I have defined a cycle as "a periodic rise and fall in the fission rate, extending over a varying number of rhythms, and ending in the extinction of the race, unless it is 'rejuvenated' by conjugation or *changed environment*."[1] This suggests the idea that it may be possible to eliminate the cyclical character of the division rate by *constantly* subjecting the organisms to a varied environment and the present investigation is devoted to this aspect of the problem. In a former paper[2] I have given an outline of my studies up to May, 1908, on the life history of *Paramecium* when subjected to a varied environment. The present paper presents the data to June 29, 1909.

II. Methods.

A "wild" *Paramecium aurelia* (*caudatum*) was isolated from a laboratory aquarium on May 1, 1907, and placed in about five drops of culture medium on an ordinary glass slide having a central ground concavity. When this organism had divided twice, producing four individuals, each of these were isolated on separate slides to start the four lines, *I–a*, *I–b*, *I–c* and *I–d* which compose this culture (Paramecium I).[3] The culture has been continued by the isolation of an individual from each of these lines almost daily throughout the life of the culture up to the present time (June 29, 1909). A record has been kept of the daily divisions of each line, and the average rate of division of the four lines of the culture and this again averaged for five- ten- and thirty-day

[1] Woodruff ('05).
[2] Woodruff ('08[2]).
[3] For further details in regard to technique see Woodruff ('05).

periods has been plotted (cf. Figs. 1, 2 and 3). Permanent preparations have been preserved at various periods in the life history for the purpose of studying the cytoplasmic and nuclear changes, if present.

The culture was carried on at the Thompson Biological Laboratory of Williams College, Williamstown, Mass., during May and June, 1907; at the Marine Biological Laboratory, Woods Holl, Mass., during July and August, 1907 and 1908; and at the Sheffield Biological Laboratory of Yale University, New Haven, Conn., from September, 1907, to July, 1908, and from September, 1908, to the present time (June, 1909).

During the first nine months of the work the culture medium was made of hay or grass; but, except during certain periods in which the culture was employed as a control for special experiments,[1] the infusion was made with hay from various localities, and different proportions of hay and water were used almost daily. Water from different sources was employed. The temperature of the infusion was always raised to the boiling point. In some cases the infusion was used as soon as it had again attained the room temperature; in others, it was allowed to stand for twenty-four hours before it was employed.

From February, 1908, to the present time, June, 1909, however, a more varied culture medium was employed. *Paramecium* will thrive in nearly any infusion which may be made from materials collected in ponds and swamps, and therefore, in an endeavor to supply as far as possible all the elements which may be encountered in the usual habitat of the organism, water was taken from ponds, laboratory aquaria, etc., together with their animal and plant life. In other words, no definite method was used in selecting the material, but it was simply collected at random from what might be the abode of infusoria, and thoroughly boiled. Probably the only condition present in the life of this culture which could not be encountered by a wild *Paramecium* was that the water had been boiled, but this was essential in the experiments in order to obviate the possibility of the contamination of the culture by an active or encysted wild specimen. Conjugation was impossible in the direct lines of the culture on account of the frequent isolations and change of medium.

[1] Woodruff ('08[1]).

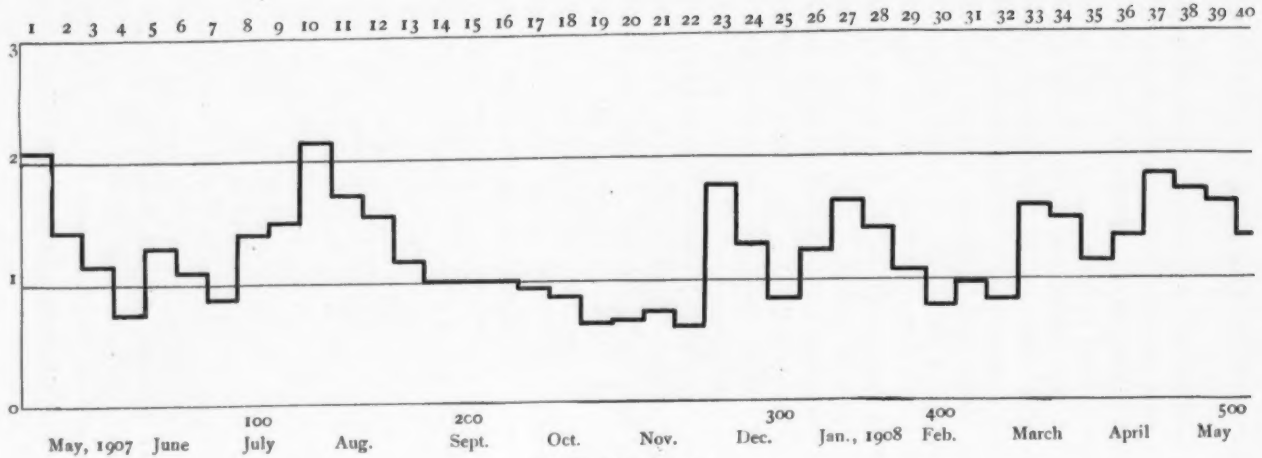


DIAGRAM I. PART I.

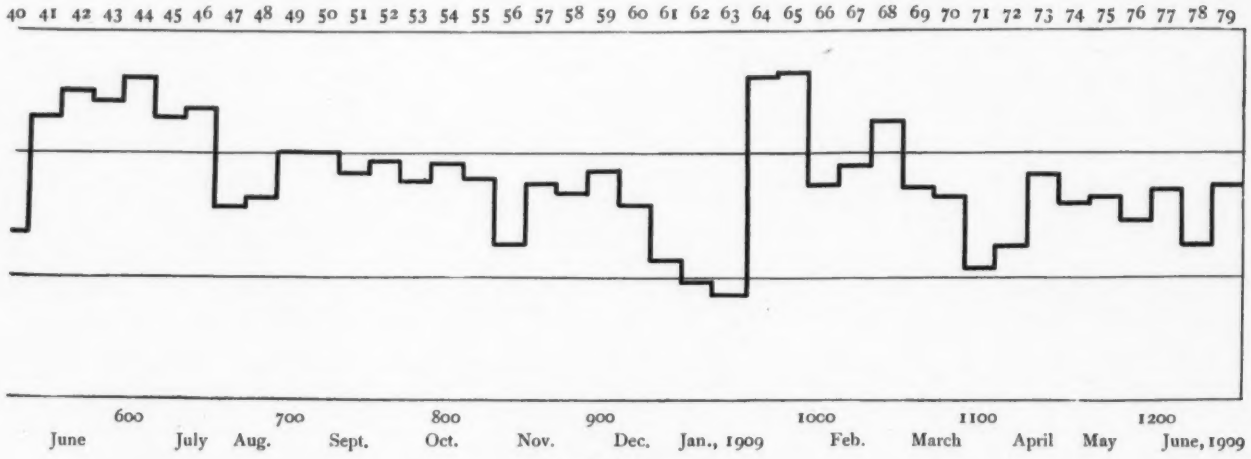


DIAGRAM I. PART 2.

FIG. 1. (Ten-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for ten-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. Above, the numbers of the ten day periods are indicated. Below, are designated the periods in which the fifteenth day of the respective months fell. The figures, 100, 200, etc., represent generations and are placed in the periods in which they were attained.

III. Description of Cultures.

Culture I has attained, during the first twenty-six months of its life, the 1,238th generation. The average rate of reproduction for the entire period has been over one and a half divisions per day, and during not a single ten-day period has the average rate fallen as low as one division in two days, while during several ten-day periods the rate has averaged over two and a half divisions per day.

Fig. 1 shows, by the familiar block method, the average rate of division of Culture I for ten-day periods. Especial emphasis is put on the character of this curve as the results of Calkins' cultures are plotted for ten-day periods. A study of this diagram shows that the life history falls naturally into two parts. The first extends from period one to period thirty, and the second extends from period thirty to the present time. These two major parts of the curve are coextensive with different methods in the treatment of the culture. The culture medium used during the periods covered by part one was very much more uniform than that used during part two; the decidedly varied environment not being employed until February, 1908. The effect of this treatment is shown in the decided change in the character of the curve of the division rate from that period to the present. The general vitality of the protoplasm is considerably higher as is shown by the fact that only once since that time has the curve for a ten-day period fallen below one division per day, and that period represents the culmination of the more or less severe treatment which the culture received when it was carried to Baltimore during convocation week. However, to the change of water and other conditions incident to the journey is apparently to be attributed the stimulus which enabled the culture to attain a short time after an average rate of nearly two and three quarters divisions per day (see Fig. 1, periods 64 and 65), the highest reproductive power shown in the life history for any ten-day period.

A study of Fig. 2, which is plotted by the same method as Fig. 1 except that the periods are of thirty-day instead of ten-day duration, also shows clearly the effect of the varied culture medium which has been in use from February, 1908. The rate

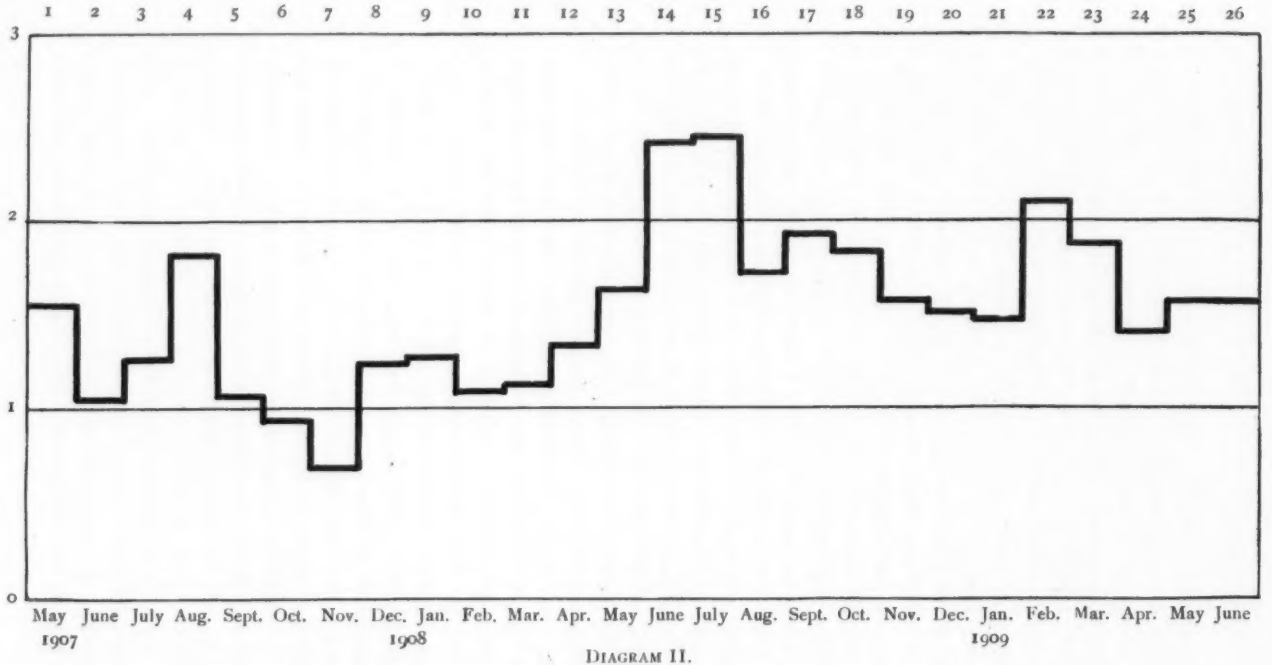

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
3
2
1
0
May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June
1907
1908
1909

DIAGRAM II.

FIG. 2. (Thirty-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for each month of the life of the culture. The ordinates represent the average daily rate of division of the four lines of the culture.

of reproduction has never again fallen as low as it was when this treatment was begun. When averaged for thirty-day periods the highest rate of division appears in June and July, 1908; the great rise in vitality which occurred in February, 1909, and is shown in periods 64 and 65 of Fig. 1 (ten-day periods) is not so conspicuous, as the average is reduced by the low rate of fission during the two periods preceding.

A similar examination of Fig. 3, in which the rate of division is averaged for five-day periods, is not so instructive because the influence of the rhythms is more clearly brought out during short periods, so that the general trend of the curve of the life history is somewhat obscured. However, when the curve is surveyed in its entirety it illustrates the fact that the vitality of the organisms, as indicated by the fission rate, has maintained a higher average since the use of a promiscuous culture medium was instituted.

In order to determine more fully the effect of a very constant environment on this same race of *Paramecium* which was being maintained on a varied environment, there was isolated from each of the four lines, on February 19, 1909, at the 1,121st generation, a second culture, designated *Paramecium* I^s. This culture was submitted to as constant an environment as was practicable, according to the general method of Calkins. There were, then, two cultures of the same protoplasm running simultaneously, one being subjected to a varied or promiscuous culture medium, and the other to a comparatively constant culture medium. As a matter of precaution, and to show if there was anything intrinsically deleterious in the medium provided for Culture I^s, its constant medium was employed at various times as a temporary medium for Culture I. This, of course, simply increased the variability of the medium of Culture I. Also, near the end of the I^s series, its medium was employed not only for Culture I, but also for two cultures of ex-conjugant paramecia (*Paramecium* II^y and *Paramecium* II^z) from an entirely different source from that of the *Paramecium* of Culture I.

The results of these experiments with a constant and varied environment on the same protozoan protoplasm are shown graphically in Fig. 4. A glance at this curve shows that the vitality of the protoplasm of Culture I^s (constant environment), as meas-

ured by the fission rate, immediately fell below that of Culture I (varied environment), and that a consistent decrease in division rate was maintained until Culture I^s died out on June 6, 1909, at the 1,159th generation, after having been one hundred and seven days, or a little more than three months, on the constant medium; whereas the protoplasm of Culture I maintained about the same general average vitality throughout the period and had attained the 1,200th generation, a gain of forty-one generations in 107 days over the I^s culture. That the death of the I^s culture was not due to some sudden and accidental inimical change in the medium is proved by the fact that the same culture medium when used temporarily for the other cultures produced no deleterious effect, and also by the character of the curve of the fission rate of the I^s culture which has a consistent general downward trend except as it is affected by the rhythms. A comparison of the I^s culture curve and the curves of Calkins' *Paramecium* cultures shows a striking similarity in character. The cycles in Calkins' A culture were of six months duration and varied between 126 and 200 generations in length. My I^s culture passed through only 138 generations, but as it actually represents only the downward slope, or second half, of a cycle of Calkins' culture, my I^s cycle is really somewhat longer than those of Calkins. This point is only of interest in that it indicates in a general way the comparative similarity of the reactions of the protoplasm of paramecia from widely different sources to the same general conditions; and because it removes the possible objection that the I^s culture died out because it had been acclimated to the varied environment, and consequently it could not withstand the change to a constant medium. Of course, this is only a formal objection at best as there is every reason for supposing that the wild paramecia with which all cultures are started have been subjected for countless generations to considerably greater variations in their environment than it is possible to supply artifically.

IV. Discussion.

Up to the present time Culture I has not completed a "cycle" and all the fluctuations in vitality, as indicated by the division rate, fall under the head of "rhythms," as previously defined by me,[1]

[1] Woodruff ('05).

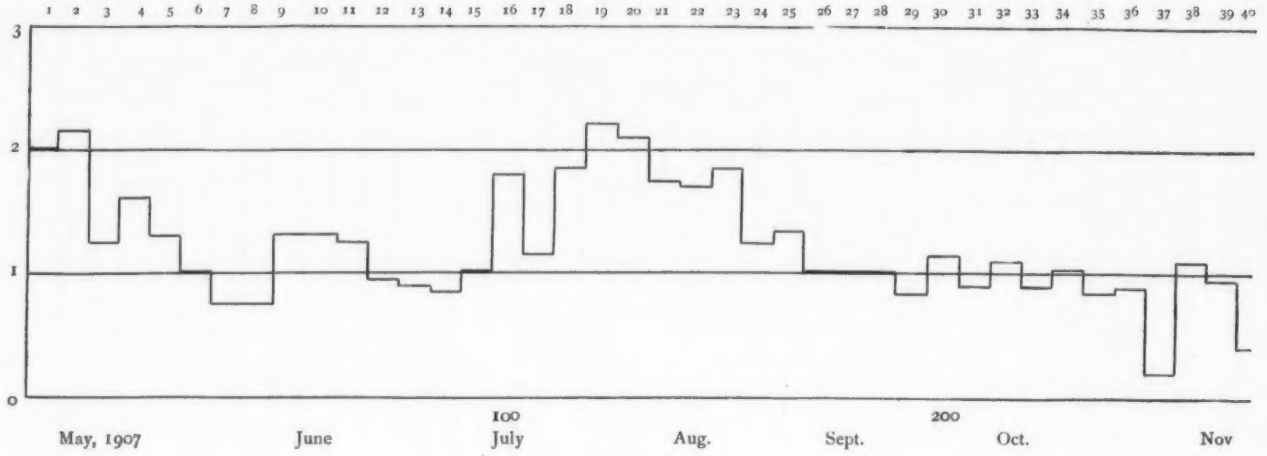
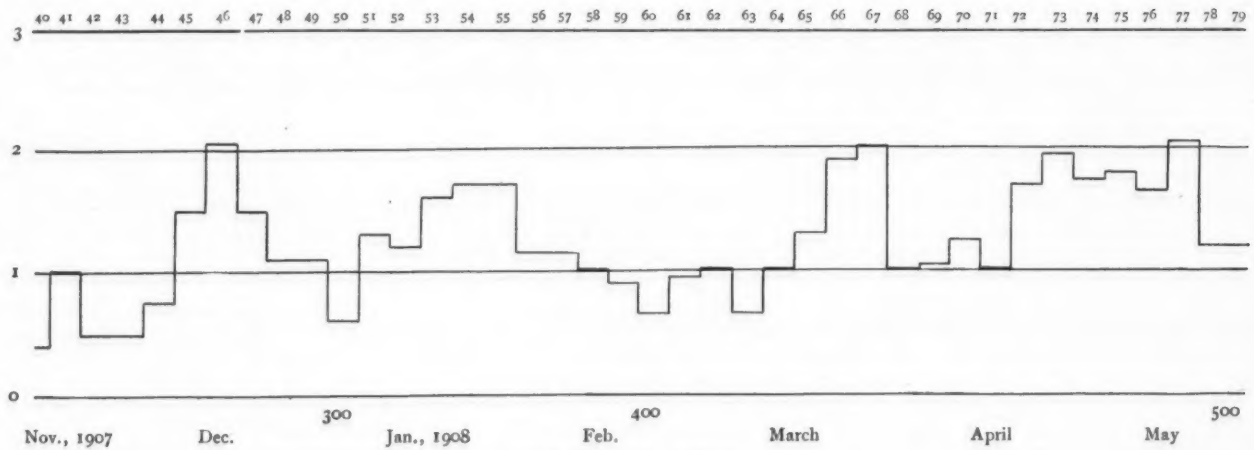


DIAGRAM III. PART 1.



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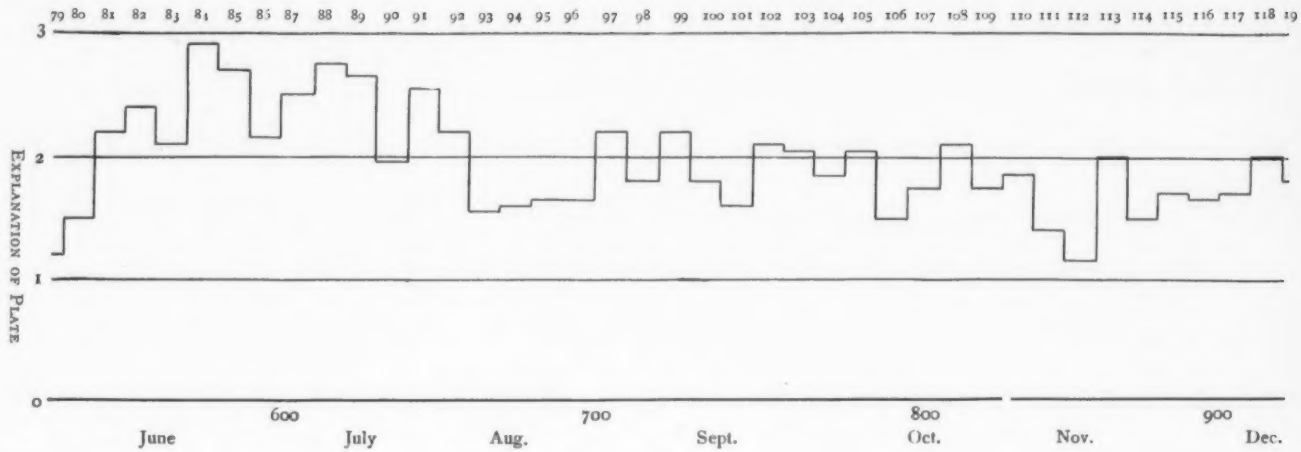

Diagram III. Part 3.

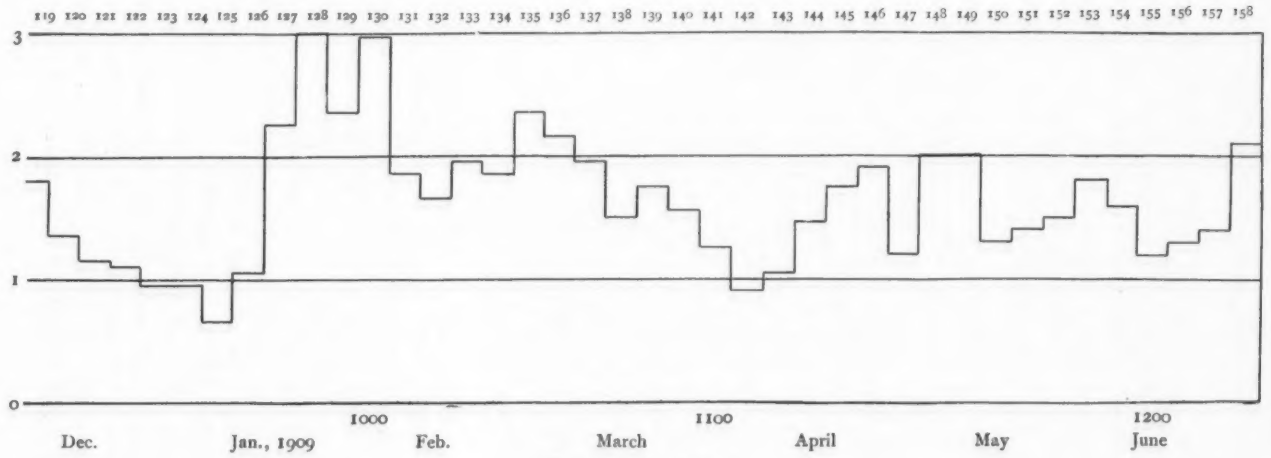

DIAGRAM III. PART 4.

FIG. 3. (Five-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for five-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. Above, the numbers of the five-day periods are indicated. Below, are designated the periods in which the fifteenth day of the respective months fell. The figures, 100, 200, etc., represent generations, and are placed in the periods in which they were attained.

viz., "A rhythm is a minor periodic rise and fall of the fission rate, due to some unknown factor in cell metabolism, from which recovery is autonomous." The rhythms are more evident when a more constant environment is maintained, as I have shown in a study of the effect of a particularly stable environment on *Gastrostyla steinii*, during the months of July, August and September (cf. Fig. 5).

Gregory ('09) has plotted the curve of a *Stylonychia* culture for five-day periods from the data of Popoff ('07), which shows that the first four of the so-called "deep depression" periods emphasized by Popoff resolve themselves into "normal rhythms from which recovery is autonomous." Gregory also points out in her own 548 generation culture of *Tillina magna* that "the curve which represents the general vitality of the protoplasm shows the normal rhythmic fluctuations observed by Woodruff."

I have previously interpreted as rhythms the tri-monthly depressions in vitality, which Calkins and the earlier workers on *Paramecium* have noted, and the results obtained from my culture of *Paramecium* seem to indicate that the semi-annual cycles of Calkins are also actually rhythms, recovery from which was not autonomous under the conditions of a constant environment. The general occurrence of rhythms in the life history of infusoria is established, I believe, but to what they are due is still awaiting discovery.

Gregory has emphasized the point that "Enough consideration has not been taken of the fact that not only does each individual vary in its degree of sensitiveness at different periods in the life history, as suggested by Towle and shown by the rhythms of Woodruff, but each individual of the same species as well as of different species has its own peculiar protoplasmic reactions. Woodruff himself has failed to consider this fact in his last paper on the effects of a varied environment on *Paramecium*. . . . He cannot logically compare his results with those of Calkins for he is not dealing with the same protoplasm. . . ." In 1905 I wrote: "My cultures lead me to believe, with Simpson, that the personal equation, if I may use that term, of the individual selected to start a culture has the most influence in determining the number of generations attained. . . . Calkins' discovery of

what he calls 'incipient fertilization' . . . would seem to bear out this point, and to show that the number of generations, or the period, over which a cycle extends, is not a point of great moment."

I have since found no reason to alter this opinion. But there must be *limits* beyond which the "peculiar protoplasmic reactions" of any individual do not extend, otherwise each would be a law unto itself and there would be as many laws as individuals. Certainly we may reasonably assume that there are limits of time, and generations, which a "cycle" (if it exists) of any particular species will not exceed. The earlier investigations apparently indicated that about three months or about 100 generations was the limit of the cycle of *Paramecium*. Calkins in his last paper extended the cycle to about six months, or about 200 generations. The present culture extends the "cycle" to more than twenty-six months, and more than one thousand two hundred generations. The longest culture carried by Calkins (Culture A) lived for twenty-three months, and attained 742 generations — but this comprised four complete cycles, the last one terminating fatally. It is necessary to contrast the cycle of Calkins' culture of about six months duration, and two hundred generations, with the life (cycle) of this culture, which is of twenty-six months duration at present, and 1,238 generations. That is, this culture shows a "cycle" twenty months longer in time, and, so far, of over one thousand more generations.

The *character* of the life history must also be taken into account. There is a marked difference in the character of the *Paramecium* curve after February, 1908, when the decidedly varied environment was begun (cf. Figs. 1 and 2). A similar difference in character is evident in the *Gastrostyla* culture when the more constant medium was being maintained (cf. Fig. 5, July, August and September), and the same is again strikingly shown in the present culture of *Paramecium* in the experiments which subjected the "same protoplasm" to a constant and a varied environment simultaneously (cf. Fig. 4).

The term cycle, as has been pointed out, is a relative one, but I think it is necessary to extend the conception of the cycle (as worked out on infusoria on constant media) to an unwarranted

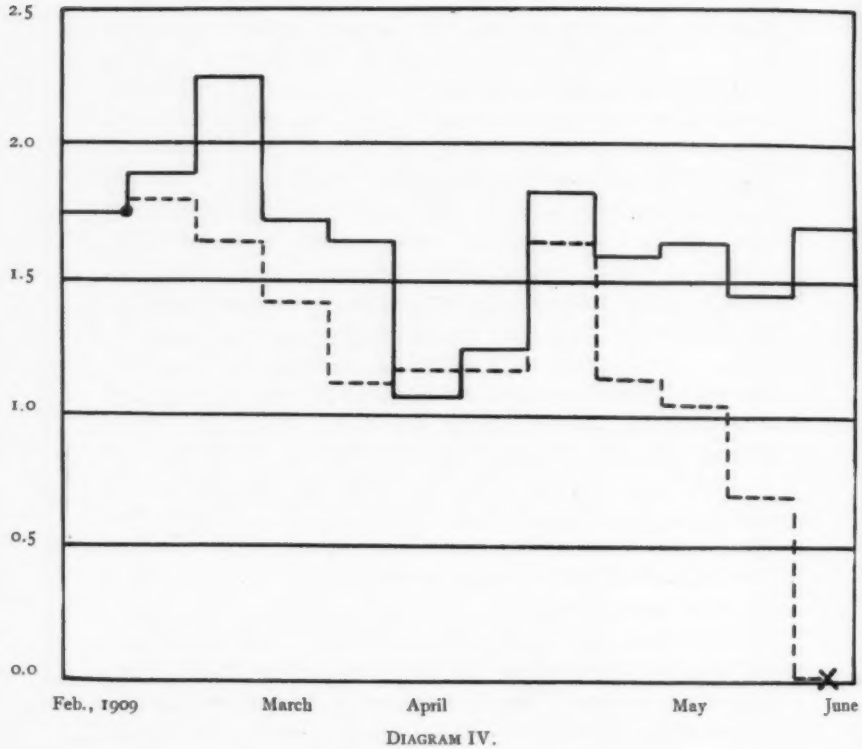

DIAGRAM IV.

FIG. 4. *Paramecium*, Culture I^a (constant environment) = broken line; Culture I (varied environment) — continuous line. ● = point of isolation of Culture I^a from Culture I. *X* = point at which Culture I^a died out. Other details as in Fig. 1.

extent if it is to be made to include the life history of the present culture. I would not suggest that the protoplasm of every wild *Paramecium* has the potential to attain over twelve hundred generations or more — undoubtedly there are strong and weak strains among infusoria as among other classes of animals. Again, it is possible that the different races of *Paramecium* which Jennings ('08) has been able to isolate may have a physiological as well as a morphological basis of distinction. It may be also that I have been particularly fortunate in my haphazard selection of culture material, so that the proper variations have been available when necessary. It is true that any particular ingredient of the infusion which might be needed would not long be available for the organisms on account of the frequent change of the character of the infusion, and Gregory has recently shown in the case of *Tillina*, and I have shown in the case of *Gastrostyla*, that daily stimulation with salts is often more efficacious than an initial stimulation. But Calkins says in regard to the second cycle of his A culture ". . . in December it was necessary to keep them on the stimulant only a day or two to get the desired result. The short treatment at this period sufficed, because they were not allowed to become weakened to the same extent as in the preceding period of depression." It is this factor which has been taken into account in this study, and it is probable that it has contributed largely to the vitality of the culture.

It must also be kept in mind that a certain amount of judgment is exercised in selecting a representative specimen for isolation. By experience one becomes quite familiar with the normal movements, shape, and general appearance of the organisms, so that it is possible to select a favorable specimen daily for the continuance of each of the lines. The precaution is nearly always taken to examine the culture again a few hours after the isolations to see how the organisms behave in the fresh culture liquid. If everything does not appear normal, a new set of individuals is isolated from the "stock" (*i. e.*, from the one, three or seven individuals left after isolation, the number depending on the rate of division during the previous twenty-four hours). Undoubtedly the process practically results in the artificial selection of the organisms which have the highest poten-

tial of division and those which are most readily acclimated to changes in their medium. Each and all of these factors may contribute to the length of the life of the culture — but after all is done the "chances" are largely against the prolonged life of the culture.

This culture suggests, then, the time-honored question whether the protoplasm of infusoria has the potential of unlimited life and reproduction, and the fundamental question as to the rôle of conjugation in the life history of these organisms. Up to the present time there has been no tendency to conjugate among the individuals of this culture, although in the "stock" cultures, consisting of the individuals remaining over after the daily isolations, there has been ample opportunity for it to take place. The daily isolations, of course, have precluded its occurrence in the four direct lines of the culture. This result agrees with those of Joukowsky on a 460 generation culture of *Pleurotricha lanceolata*, Gregory on a 548 generation culture of *Tillina magna*, and Woodruff on an 860 generation culture of *Oxytricha fallax*, on a 448 generation culture of *Pleurotricha lanceolata* and on a 288 generation culture of *Gastrostyla steinii*. Maupas secured no conjugations in his cultures of *Stylonychia mytilus* and *Oxytricha* sp., though his other series yielded plenty of syzygies. That the infusoria do conjugate is, of course, a matter of common observation; but I believe these results indicate that the phenomenon is not so frequent in the life history as is generally believed. A daily examination of twenty hay infusions, made up by several different methods, has not shown a single case of conjugation among the hypotrichous forms present either at the top or bottom of the jars. In fact, not a single syzygy has been observed in any species except *Paramecium*, and in this form conjugation has been very rare. However, a sudden transference of the paramecia from the comparatively constant culture medium of a hay infusion to a different medium has produced marked epidemics of conjugation. It is just possible that a constant medium is necessary for the so-called miscible state (Calkins) to develop, and that this becomes functional on transference to a decidedly different medium. If this is so, it may account for the absence of conjugation in my paramecia series on a varied medium, and

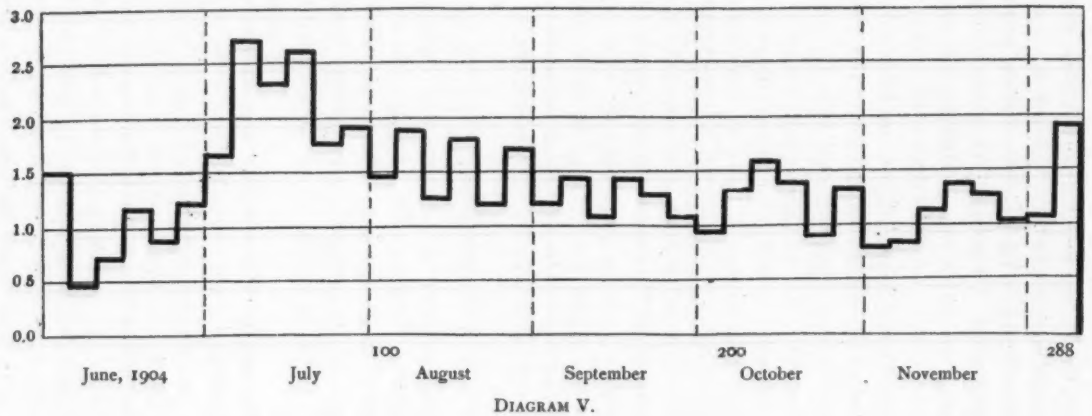

DIAGRAM V.

FIG. 5. Complete history of *Gastrostyla steinii*, Culture A, averaged for five-day periods. Method of plotting is the same as in previous diagrams.

its prevalence in Calkins' cultures on a constant medium. This idea is not supported by my hypotrichous cultures which were carried on a constant medium and still did not develop a tendency to conjugate even when the culture medium was varied in some special experiments. It is highly probable, however, that the superficial conditions which induce conjugation may vary in different species.

Periods of marked physiological depression have not appeared during the first twenty-six months of the life of the *Paramecium* I culture, but well-defined morphological changes have taken place. I shall not discuss these cytological changes at present, as I believe it is advisable to wait until the culture is terminated naturally, or by accident, so that all the data from the complete series may be discussed in its entirety. It is clear, however, that the relation of the rate of division to the so-called "normal" condition of the nuclei of *Paramecium* is not supported by this culture, as decided nuclear changes apparently do not affect the general vitality of the organisms. It may be noted further, that not a single monster due to incomplete or otherwise abnormal division has occurred in the entire 1,238 generations.

V. Conclusions.

The experimental study of the life history of infusoria has so far clearly shown that:

The protoplasm of these organisms, when subjected to a comparatively constant culture medium, passes through long cyclical changes in vitality which finally result in the death of the organism.

The protoplasm may be "rejuvenated" by suitable changes in the culture medium (stimuli) at critical points in the cycle, and thus be enabled to resume active reproduction for a longer period.

The essential fact brought out by this study is that:

The protoplasm of the individual *Paramecium* isolated over two years ago to start the culture has had the potential to divide (so far) over one thousand two hundred and thirty times at an average rate of more than three divisions every two days, and the representatives of the untold millions of its progeny which are

still in captivity give every indication of being in as normal physiological and morphological condition as their ancestor. This suggests that when the protoplasm is constantly subjected to a suitable varied environment the cycle may be greatly prolonged and probably entirely eliminated—the fluctuations in vitality not transcending the rhythm.

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ON THE METHOD OF CELL DIVISION IN TÆNIA.[1]

A. RICHARDS.

Within the last few years the question of the significance of amitosis has pushed its way forward with renewed activity. From the classic views of Ziegler and vom Rath, Flemming and others that amitosis may be expected in unicellular organisms, in degenerating and senescent cells, and in highly specialized and pathological tissues opinion in some quarters has departed widely.

On the one hand the tendency has been to narrow this view. In many of the Protozoa mitosis has been found quite general, at least in some stage of the life cycle, while in certain Rhizopods, as *Arcella* and *Euglypha*, where direct division was formerly thought to be the means of reproduction, it is now known that mitosis is the common method. In the case of highly specialized cells numerous examples are reported in which careful study has shown mitosis as the chief means of division. An example of this tendency is seen in Strasburger's work on the tapetum cells. He found that while the period of mitotic division was very short it was sufficient to account for all the observations that had hitherto been explained on the basis of amitosis. Again the application of improved cytological methods of fixing and staining have thrown into disrepute, to a large extent, the old view of the occurrence of direct division in pathological tissues; indeed the phenomenon of reduction has been described in cancer cells. On these lines of research, then, the tendency has been to limit our notions of the rôle of amitosis in nuclear and cell division.

On the other hand more recently a new line of reasoning has been developed, perhaps more rapidly than the facts warrant. This new line, of which Child is the chief, although not the first exponent, is to the effect that direct nuclear division occurs in rapidly dividing cells and in cases in which an orthodromic or acyclic process is involved. In this connection the statements of

[1] Laboratory of Zoölogy, University of Wisconsin, June 4, 1909.

several writers that mitosis may follow amitosis are to be noted in which they cite cases of direct division in the maturation and prematuration stages of various forms. To meet these claims we shall doubtless have to revise somewhat our ideas of the meaning of amitosis, but at present the progress toward such a revision seems to have overstepped the bounds of conservatism.

Among the older workers on this line are Meves, Preusse, and Pfeffer. Meves found amitosis to occur in the early stages of spermatozoön formation in *Salamandra* in the autumn followed by mitosis in the spring, but some of these cells have since been shown to take no part in the formation of spermatozoa. Preusse's work has been much quoted in this connection. He found amitosis in the ovaries of Hemiptera. However, a reinvestigation of this case by Gross in 1901 served to bring it under the theory of Ziegler and vom Rath. Gross showed that this method of cell division did occur but much less widely than described by Preusse. Its occurrence is restricted to two kinds of cells, degenerating and secretory; this, of course, proved that he was dealing with a special case under the old theory. Pfeffer's work on *Spirogyra* has been discredited by Nathansohn; in fact, opinion among botanists is decidedly adverse to the view that amitosis may be followed by mitosis in a single nucleus. This opinion is expressed by Strasburger in his recent summary of the individuality question.

Working on the spermatogenesis of the sparrow in 1900, Loisel saw nuclei which began division by amitosis and later continued by indirect division. He says that the amitosis was not a sign of degeneration; but again, he shows that the greater part of certain spermatocytes and spermatids degenerates. To reach a safe conclusion in this case one must needs know the relation between amitosis in the sex cells and degeneration in the sex products. Degeneration on the part of spermatozoa in the Hemiptera has been traced by Morgan and by Miss Stevens to the absence of a single chromosome. If cells lacking a single chromosome degenerate, certainly one would expect degeneration in cases where part of the sex cells had previously divided by as indifferent a method as amitosis seems to be.

Especial importance has been attached to cases of amitosis in

regulatory growth. G. T. Hargitt was the first to suggest this for hydroids. Child's work on *Tubularia* and *Corymorpha* supports this suggestion. In the growth and regulation of *Planaria*, Bardeen and Child have reported amitosis. However, the figures of Bardeen are far from conclusive, and it is very questionable whether they justify the opinion that amitosis is the method of division here. Child has also worked on various other forms among both vertebrates and invertebrates. In several of them his evidence is lacking in some respects. Reference to his work on the cestodes will be made later.

A few workers have described amitosis in the cleavage of the egg and in the early embryonic development of several forms. Hargitt failed to find mitosis of the egg up to the sixteen-cell stage, working on *Clava leptostyla*. Similar results obtain for *Eudendrium* and *Pennaria*. Beckwith, however, has recently shown that his results were due "simply to the fact that the eggs were not obtained at the right time of day. In eggs collected at the proper time (4 to 6 A. M.) there is no difficulty in proving the typical stages of maturation and fertilization." "Maturation and the early cleavages take place by mitosis and not by amitosis." Hickson and Hill have also studied cœlenterate eggs. Hill in his account of *Alcyonium* oögenesis shows that no polar bodies are extruded, no chromosomes are present, the female pronucleus divides irregularly by amitosis and then disappears, and that probably the first cleavage nucleus is formed from the male pronucleus. The evidence is not complete and the case should certainly be reinvestigated. H. L. Osborne described cases of amitosis in the food-ova of *Fasciolaria*. His results have been corrected and enlarged upon by Glaser. The work of Glaser seems to deserve the most careful consideration in regard to this problem; its bearing on the investigation herewith undertaken is only general, however. Further work on embryos has been done by Child, previously mentioned, and by Patterson on the pigeon's egg. The observations of the latter, while much more extensive than those of Child on the chick embryo, are in agreement with them. Stoeckel thought binucleate ova in man are the result of amitotic divisions. Fick's opinion on the subject of amitosis as expressed in his survey of chromosome hypotheses is based

upon the work of Child and Hill and upon his own *a priori* conclusions. He offers nothing new on the problem.

Direct division has been described in other cases of theoretical importance but those mentioned above are perhaps the most significant.

Methods.

My investigation on the problem of amitosis was suggested by Dr. S. J. Holmes, to whom I owe much for direction during the progress of the work. I have received numerous suggestions from various other workers in the University of Wisconsin, all of which are gratefully acknowledged. My thanks are also due Drs. Grove and Meek, of the Pharmacology and Physiology Departments, for their assistance in collecting material.

Specimens of tape-worms were secured chiefly from dogs. A considerable number of cats was examined, but only one furnished material. The specimens were nearly all fixed in Flemming's fluid, which proved quite satisfactory. Those taken from the cat were fixed in Zenker's fluid to be used with Mallory's connective-tissue stain.

A variety of staining methods was used. Flemming's tricolor stain did not give sufficient sharpness of detail to be of much value. Iron hæmatoxylin is in general satisfactory, but it is to be noted that the nuclei do not differentiate as readily as in many other tissues. The fact that they do not decolorize readily and often do not show their contents clearly must be borne in mind when considering the significance of indentations of the nuclear membrane. Delafield's hæmatoxylin decolorized in acid alcohol gave excellent results. My greatest success, however, in staining this material has been by the use of Kernschwarz with Lichtgrün as a counterstain. Lichtgrün is by far the best stain for cytoplasmic structures that I have tried, its only drawback being the ease with which it fades out.

Two genera and three species of tape-worm have been used: *Tænia marginata* (Batsch), *Tænia serrata* (Goeze), and *Dypilidium caninum* (Leuckart). The last of these is the most favorable for cytological work, but I have only a few specimens of that genus. Even here the nuclei are quite small and not entirely satisfactory owing to technical difficulties. I must protest against

the balancing of results obtained from such unfavorable material as that which the cestodes offer against such favorable objects for cytological study as, for example, the Orthoptera. In the cestodes which I have studied, the cells, except the oöcytes, are much smaller than the insect cells, do not stain as readily, and are often obscured by great masses of intercellular material.

Aim.

My aim in this investigation was to obtain definite evidence as to the occurrence of amitosis in cestode tissues. Observations were begun with the hope of bringing into line, in a small measure at least, the account of cell division in this group with the results generally obtained by workers on other forms. Lack of time has prevented the investigation of many of the secondary questions that have arisen. Thus no attempt has been made to give details of chromosome behavior or structure, and the observations have been limited to the method of cell division in the process of oögenesis and in the growth of somatic cells. The discussion, however, includes occasional reference to related questions.

Observations.

Oögenesis. — The female sex cells in the cestodes in question are by far the largest cells in the body. They are in general round with a relatively large nucleus. The cytoplasm is fibrorecticular and to a certain extent granular. Occasionally large dark granules appear, as in Figs. 15, 16 and 17; their nature has not been definitely made out, but they may be yolk nuclei or, perhaps, nothing more than aggregations of smaller cytoplasmic granules. Frequently they serve to obscure the process of mitosis.

No cell organs are located in the cytoplasm of the resting mother cells; but, at some time during the development of the ovarian egg, a mass, probably of yolk, appears there. I have not observed any regularity in the formation of this mass, for some of the early oögonia have it, while it is not present in some oöcytes. The masses, of course, vary in size. Those which are newly formed have a close resemblance with certain stains to a "nebenkern," and, in fact, have been so called. They

were first described by Sommer in 1874, and named by him "Nebendotter." This expression, which has the claim of priority, seems unobjectionable except on the score of bringing a foreign word into English; no suitable translation has been suggested, however. On the other hand, the body is not a true "nebenkern," and to call it by that term is a misuse of the word.

Fig. 1 is an early oögonium from *T. serrata*. Here the "Nebendotter" appears as an egg shaped body of even consistency stained darker than the nucleus although lighter than the

1 2

FIG. 1. Oögonium, stained with iron hæmatoxylin, showing "Nebendotter," and nucleus with chromatin reticulum.

FIG. 2. Oögonium showing same structures as Fig. 1, but from a much later generation.

surrounding cytoplasm. Its reactions to various stains deserve metion. With iron hæmatoxylin it stains readily, appearing as a dark homogeneous mass even after a great deal of extraction of the stain. The nucleus and cytoplasm may be entirely decolorized and the "Nebendotter" still show as a dark body, a fact which led to confusion during the early part of my study, as the nucleus was overlooked and the "nebendotter" taken for it. The true state of affairs was not revealed until I had used another method of staining when the appearances which with iron hæmatoxylin had misled me were explained and the structure of the cells became clear. The new reagents were Kernschwarz counterstained with Litchtgrün. Kernschwarz is a weak stain affecting only the nucleus. In my preparations I have seen no trace of it in the cytoplasm or in the "Nebendotter." Lichtgrün stains both nuclear plasm and cytoplasmic structures. The result, then, with this method of staining is as follows: chromatin and nuclear

reticulum, blackish; nuclear plasm, very light green; general cytoplasm, dark green, fibro-reticulated; "Nebendotter," homogenous "cheesy" green. This last appearance is very difficult to describe but is recognized very easily.

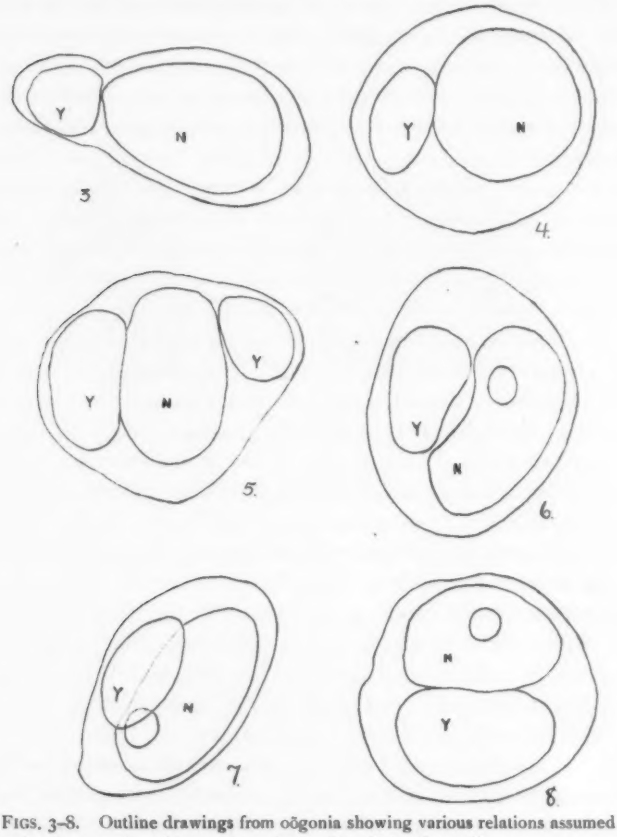
The "Nebendotter" as such has not been described by Child. One is compelled to suspect that the same appearances which confused me may have misled him. Occasionally a constriction is seen in a "Nebendotter" or there may be two or more distinct yolk masses in a cell. More often, especially in the case of hæmatoxylin slides not well decolorized, the "Nebendotter" does not look unlike a dividing or divided half of the nucleus. Child figures cases in which one half of the nucleus stains darker than the other half. Is this darker half perhaps a "Nebendotter"?

To illustrate the above facts a series of outline drawings is given. They were made with the aid of a Zeiss No. 5 ocular and a Leitz one twelfth objective (oil immersion). In each case *n* represents the nucleus and *y* the "Nebendotter." In studying these figures one can easily see how refractive properties may have obscured the boundaries between the various parts. All figures in this series are of resting cells such as are shown in Figs. 1 and 2. Figs. 3 and 4 resemble cases of unequal constriction of the nucleus, Fig. 8, of equal constriction, and Fig. 5 a nucleus divided into three parts by amitosis. Fig. 6 suggests that division began at the center and progressed outward. Compare these figures with those from Child's paper on oögenesis (6): Fig. 1 with his Fig. 29; Fig. 2 with his Fig. 9, *b*; Fig. 3 with his Fig. 8, *b*; Fig. 6 with his Fig. 11, *A*, *a*; Fig. 7 with his Fig. 13, *A*; and Fig. 8 with his Fig. 10, *A*. The similarity is very suggestive.

Resting nuclei are seen in Figs. 1, 2, and 9. Fig. 9 is lacking in a "Nebendotter." The nuclear plasm in the resting condition seems homogeneous throughout and takes a very light stain. The nuclear membrane is a very delicate structure, showing as a thin line in some cases, while in others its location is marked only by the inner edge of the cytoplasmic reticulum. A nucleolus is usually present in the early stages of cell formation; it takes a very light stain in some cases resembling the "Nebendotter." I have never seen a divided or dividing nucleolus.

With regard to their chromatin content the nuclei of *Tænia*

differ from those of *Monieza*. Child states that "the only deeply staining portions of the nucleus up to this time (end of oögonial division period) have been the nucleolus and frequently a few other granules." In another place he says that the nuclei do



FIGS. 3–8. Outline drawings from oögonia showing various relations assumed by the nebendotter and nucleus. *N*, nucleus; *Y*, "Nebendotter."

not contain any definite reticulum. That his statements do not hold for *T. serrata* may be seen from my Figs. 1, 2 and 9. The chromatin content is small in amount, but it can be seen in definite masses which are scattered over the periphery of the nucleus and which are connected by definite strands of linin. The reticu-

lar character of the chromatin and linin is clearly shown with Kernschwarz and Lichtgrün. This description answers not only for late oögonia but also for the early ones and for the oöcytes. Fig. 9 is an early oögonium.

Fig. 10 illustrates the early condition of the spirem stage. Oögonia in this stage and slightly later are very numerous in certain lots of material. In other lots I find many resting stages. Anaphases and telophases, too, are not difficult to find but metaphases are conspicuously absent. This probably means that the metaphases are of short duration. It also indicates a fact which I believe to be very pertinent to the question of the frequency of mitoses; namely, a periodicity[1] with regard to

FIG. 9. Resting oögonium with a definite chromatin reticulum; no "Nebendotter" present.

FIG. 10. Oögonium; very early prophase, spireme formation beginning.

FIGS. 9–17. From cells stained with Kernschwarz and Lichtgrün.

the divisions. It is well known that physiological factors may govern the time of mitotic divisions. A case in point is that recently described by Beckwith, previously mentioned; likewise, in certain insects and in many plants mitosis occurs at night only. The fact that many nuclei from one lot are in the same stage of division indicates, I believe, the effect of some physiological factor. What that factor is, I can only conjecture. Perhaps mitotic periods may occur only after a more or less prolonged fast on the part of the host, for then the energies of the parasite are not directed towards the assimilation of food.

During the maturation period the regular course is followed.

[1] This expression is not intended to imply that a definite amount of time intervenes between successive periods; they may recur at irregular intervals depending on some physiological factor.

Figs. 13 to 17 illustrate the process, but no attempt is made to give even a meagre outline of the behavior of the chromosomes in this period. However, attention is called to the appearance of the spindle. While spindle formation is quite regular, the achromatic fibres do not stain well and frequently the entire structure is overlooked. This fact taken with the smallness of the chromosomes and the prominence of the large cytoplasmic granules may well serve to veil the process of mitosis. In many cases one does not, at first, distinguish between the chromosomes and the

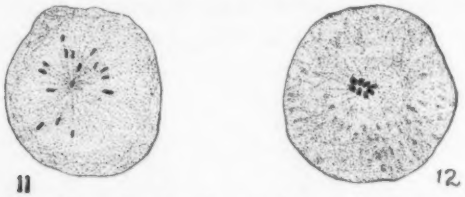


FIG. 11. Oögonium; polar view of an anaphase. This figure and the next are from early generations of oögonia.

FIG. 12. Oögonium; polar view of a telophase.

cytoplasmic granules, so nearly alike may they appear. While Fig. 15 shows clearly its mitotic character, the cell from which it was drawn was overlooked for a long time.

Fig. 17 is a case which suggests an "endogenous" division. According to Child, an endogenous division is that of a nucleus into two nuclei within the old nuclear membrane. Upon a superficial inspection the cell in question seemed to be in process of such division as shown by the outline in Fig. 18. Careful study, however, revealed the mitotic nature of the division. It is a late telophase with the chromosomes disintegrating; remnants of asters may be seen, as can a Zwischenkörper.

The appearance of this cell suggests the question of the relation between nuclear and cell division. Botanists have recognized the distinction between the two processes much more generally than have zoölogists. The latter have been accustomed to regard nuclear division as a sign of immediate cell division. Very often this is not the true state of affairs, for nuclear division may never be followed by cell division (Marshall), or a considerable period of time may elapse before a cell plate is formed. Fig. 17 shows

no sign of cell division although mitosis is almost complete. This is not an infrequent occurrence; actual nuclear division and certainly cell division may lag well behind spindle and chromosome division. Herein lies a fruitful cause for misinterpretation. A nucleus in which chromosome division has been completed would give every appearance of direct division upon constriction and subsequent division. Two nuclei in a single cell which had not begun to form a cell plate would also be misleading.

No true case of amitosis has been observed in the egg cell formation of the *Tænias* upon which I have worked.

Somatic Tissues. — The somatic tissues of tape-worms inside the cuticle include muscle fibres, excretory and genital organs, and a primitive nervous system. Surrounding all of these organs and filling all interstices is a large mass of parenchyma. A detailed study of all of these structures has been made by Child for *Monieza*. He reports that many cases of amitotic division occur and that illustrations of this fact might be multipled indefinitely.

I have made no investigation of the method of cell division in the excretory or nervous systems of *Tænia*. There is no reason

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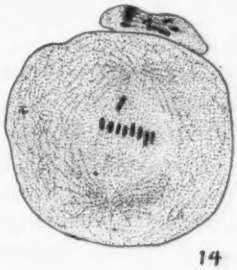

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FIG. 13. First oöcyte; late metaphase.
FIG. 14. Second oöcyte; equatorial plate stage, first polar body dividing.

to believe, however, that the method of division in those organs differs from that of the genitalia or of the muscle fibers. We have in all of these systems cells that are specialized to a high degree. Even if amitosis be found here where rapid growth may be taking place that fact loses significance when the degree of specialization is considered. The various cell generations of a differentiating tissue differ from earlier generations only in a gain

in specialization and a loss in reproductive potentiality. Rapidity of division in these cases is a negligible factor. We are simply dealing with a special case under the old theory, if amitosis be found to obtain here. Amitosis, then, may well be expected in these systems.

The facts observed in the genitalia of *Tænia* do not bear out fully that expectation. The structure of the genital ducts and organs can be made out clearly and nuclei, cytoplasm, and intercellular substance seen. Yet cases of amitosis have not been demonstrated. On the other hand, a mitotically dividing nucleus is found only rarely. This may mean that mitosis is of very

FIG. 15. Second oöcyte; early anaphase, spindle stained very lightly. Figs. 15–17 show the large cytoplasmic granules.

FIG. 16. Second oöcyte; telophase.

short duration, or more probably, that it is of short duration and occurs in waves; or, again, it may indicate that the nuclei divide amitotically. But whichever interpetation we may accept we do no violence to the theory of Ziegler and vom Rath.

The muscle-cells also furnish only negative evidence of amitosis. They are large, spindle-shaped cells from which contractile fibers extend. The cytoplasm is densely reticulated, rarely exhibiting the vacuolated structure described by Child (8). The quantitative relations of cells and fibers at different periods of development are of interest. Relatively more muscle cells are present in a young proglottid than in an old one, but the muscle fibers are much more developed in the later stages. The significance of this relation, which agrees closely with a similar parenchymal relation, will be discussed later. While satisfactory evidence as to the usual method of cell division has not been obtained, the observations on the material at hand favor mitosis as typical.

The meaning of amitosis in the parenchyma is of quite different import from that in specialized tissues. Parenchyma is a tissue from which others are derived; it is neither highly specialized, degenerating or pathological. The occurrence of amitosis here, therefore, would not be in line with the old theory.

Concerning the nature of parenchyma there has been much

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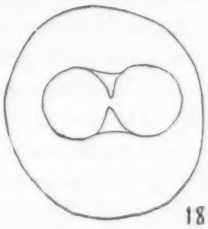

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FIG. 17. Second oöcyte in which mitosis is nearly complete, yet no sign of cell division. The daughter nuclei are being constricted apart and the chromatin masses are disintegrating. Zwischenkörper present.

FIG. 18. Outline drawing of Fig. 17. It gives the appearance of an "endogenous" division by amitosis. Fig. 17 shows that appearance to be entirely superficial.

controversy, but it is beyond the scope of this paper to enter into a discussion of the question or of the literature regarding it. In connection with an account of the method of cell division, however, a few observations concerning it cannot be avoided. The older view held by Leuckart and his followers was that the parenchyma develops from rounded and polyhedral cells, the latter sending out processes which interlace about the former. Moniez and his followers offered an opposed view. Opinion has by no means become settled even now. Child speaks "of the parenchyma cells if the syncytium which composes the parenchyma can properly be said to be composed of cells."

Parenchyma consists of calcareous bodies and irregularly shaped cells lying in a mass of material which seems to be intercellular. Of these, the first do not enter into the question under consideration, and the intercellular matter only figures as a ground substance in which the cells are imbedded. The cells vary in shape from elliptical and spindle-shaped to an irregular form with many protoplasmic processes. In size there is also much variation, due, largely, to the variation in the amount of the

cytoplasm, for the nuclei do not exhibit any such striking quantitative differences as does the cytoplasm. The cells do not seem to form a syncytium of which the ground substance is a part, as some writers have stated. The evidence seems to me to indicate that the ground substance bears a relation to the parenchyma cells similar to that borne by the intercellular matter of connective tissue, for example, to the connective-tissue corpuscle. That the parenchyma cells have definite boundaries is brought out clearly in the Lichtgrün preparations. I have seen no evidence for thinking the ground substance continuous with the cytoplasm. The nuclei of the cells show uniformity of structure as well as of size; they have a chromatin reticulum and usually a nucleolus. The parenchymal cell is thus seen to be a definite structure with a typical nucleus and a varying amount of cytoplasm.

Fig. 19 is a resting parenchyma cell of characteristic appearance. The cytoplasm is drawn out into strands upon the number

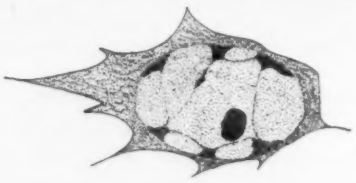

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FIG. 19. A typical parenchyma cell in the resting condition. This cell is from a proglottid in which the sex organs are only partially developed.

and size of which depends the width of the cytoplasmic band about the nucleus. Parenchyma shows regional differentiation in the relative amount of ground substance and cells and in the modification of the cytoplasmic parts of the cells only. In the younger cells the cytoplasmic strands are less numerous and frequently extend only a short distance. In older regions more cytoplasm is drawn out into the strands, leaving but a thin layer about the nucleus.

Professor Child has assumed through all his work that the absence of mitotic figures in tissues known to be growing rapidly is evidence of the occurrence of division by amitosis. This assumption is, of course, based on good *a priori* reasoning, but I

do not believe that it is borne out entirely by the facts. In *Tænia* the parenchymal cells are relatively less in number in the old tissue than in the younger, a relation which is only partially explained by the fact that other kinds of cells develop from the parenchyma. On the other hand, the ground substance in the young proglottid is more spongy and less in amount than in the older portions of the animal. These observations show that the growth of the parenchyma is not due alone to cell multiplication but also to the formation of new intercellular matter and to the greater development of the cytoplasmic strands. The growth of the cestode soma is due chiefly to the growth of the parenchyma and of the muscle cells and fibers. Other tissues, except the sex products, are practically negligible in accounting for increase in size. With regard to the growth of the muscle fibers a similar condition obtains in them as in the parenchyma, as is shown above. Thus we find growth depending not so much on the increase in the number of cells as in the increase in amount of products of cellular activity, that is, in fibers and intercellular material. Therefore, the rapidity of growth in the cestode body does not necessarily postulate a large number of dividing cells.

As to the method of cell division little can be said. No traces of amitosis appear. Cases of several nuclear divisions in a common cytoplasmic mass such as are figured by Child (11) for *Monieza* are not to be seen in *Tænia*, nor is any explanation of them afforded by the latter genus. I have also observed no cases of mitosis. The evidence at hand, I believe, does not warrant any conclusion as to the method of cell division in the parenchyma of this form.

Summary.

1. Amitosis has not been found to occur in the oögenesis of the cestodes studied.

2. All observations on the process of oögenesis point to mitosis as the usual method of cell division.

3. The presence of a "Nebendotter" which has peculiar staining properties gives a misleading appearance of amitosis.

4. Maturation is of the typical form.

5. Indirect evidence strongly suggests that physiological factors influence the frequency of mitotic divisions.

6. Nuclear divisions are not always followed immediately by cell division.

7. Only negative evidence as to the method of division in somatic structures has been found; there is no satisfactory evidence of amitosis and mitosis is not abundant.

8. Rapidity of growth in the somatic tissues of the cestodes body does not necessarily postulate many division figures.

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